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A Comprehensive Evaluation of Three Selected Species of Cultivated Grasses

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A COMPREHENSIVE EVALUATION OF THREE SELECTED SPECIES OF CULTIVATED GRASSES

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Abstract

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Under the supervision of Professor L. D. Kamstra

Studies were made to determine if *in vitro* methods, including digestibility, could be used as selection criteria by the plant breeder and to predict animal utilization of forages. Grasses used in the study were Manchar and Sac bromegrass (*Bromus inermus* Leyss), Greenar and Oahe intermediate wheatgrass (*Agropyron intermedium*) and Siberian and Nordan crested wheatgrass (*Agropyron desertorum*). *In vivo* (conventional digestional trial) and *in vitro* comparisons were made to determine if the same differences in digestibility could be demonstrated between varieties of the three species by both methods. The forages were harvested at 50% head emergence and 14 days thereafter. *In vivo* digestibility differences between varieties within a species were small and nonsignificant. However, intermediate wheatgrass varieties differed significantly when closely controlled *in vitro* techniques were used. It would appear that *in vitro* techniques were at least as effective as *in vivo* methods in evaluating relative differences in digestibility of grass varieties. A highly significant correlation ($r = 0.89$) was shown between the methods.

In an effort to determine if varietal differences were consistent, chemical analyses and *in vitro* digestibility determinations were made on forage varieties sampled at six day intervals throughout

the 1966 and 1967 growing seasons. With the increasing level of acid-detergent fiber and acid-detergent lignin, *in vitro* cellulose and dry matter digestibility declined significantly. Differences between varieties within a species, however, were small and any significant differences shown would be unimportant. The above differences between varieties were not consistent throughout the season, indicating that multiple sampling dates should be used in plant selection programs.

The most severe test of *in vitro* evaluation methods involved plant fraction separation. In determining the effects of anatomical changes on chemical composition and *in vitro* digestibility of grasses, plants were separated into leaf blade, leaf sheath, stem and seedhead fractions. Leaves contained less acid-detergent fiber and lignin and were more digestible than the other fractions with few exceptions, while stems contained the most acid-detergent fiber and lignin and were lowest in digestibility. Leaf sheath values for acid-detergent fiber, acid-detergent lignin and *in vitro* digestibility were intermediate between leaves and stems but resembled stems more closely than leaves. Seedheads were variable in composition and digestibility apparently due to development of the endosperm followed by shattering of the ripened seed. Thus, selection by the plant breeder for forage varieties having stems and sheaths which are high in digestibility would appear more beneficial than selection for leafiness since these two fractions composed nearly 70% of the plant dry matter shortly after heading. For example, this was shown by the lower digestibility of intermediate wheatgrass varieties which contained a larger percentage

of sheath and stem tissue than the other species. Below normal precipitation during the early part of the 1967 growing season warranted a study of drought effects. Certainly climatic conditions are factors to be considered in plant selection. The results showed that plants grown on the drier soils contained significantly less acid-detergent fiber and significantly more *in vitro* digestible dry matter and cellulose and produced lower yields of dry matter per hectare.

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INTRODUCTION

Evaluation of forages by the plant breeder has consisted primarily of assessing their resistance to disease, tolerance of insects, winter hardiness, chemical composition and yield. Little consideration has been given to digestibility since suitable methods have not been available for determining the nutritive value of a large number of breeders lines of forage varieties. This has often led to the release of varieties without determination of an estimate of the actual value or yield potential as expressed by digestible dry matter. Until such methods are available, new varieties will continue to be released with little information relative to their feeding value as pasture or harvested feed.

The above factors along with the increased interest in breeding new, high yielding varieties of forage have created a need for methods which the plant breeder can utilize along with present selection criteria to select highly digestible plants early in the propagation of new varieties. Owing to necessity these methods must rely on laboratory procedures since adequate material for animal evaluation is usually not available.

It appears from a review of the literature that various methods are now available which could be adapted for use by the plant breeder for evaluating grasses. The development of new, less laborious chemical analysis procedures for determining lignin and fiber content along with the development of *in vitro* dry matter and cellulose digestibility procedures offer considerable promise for use by the plant breeder.

The most effective method or combination of methods is yet to be ascertained and becomes one of the primary considerations of this study.

The purpose of this study was to determine if laboratory methods such as cellulose, acid-detergent fiber, acid-detergent lignin, *in vitro* dry matter, cellulose and protein digestibility could be successfully used by the plant breeder to evaluate differences between breeding material such as varieties of forage within the same species. The data obtained using laboratory procedures were then compared to results obtained in a conventional digestion trial using sheep. Three species of grass were used with two varieties within each species. This investigation was conducted with the following specific objectives:

1. To compare the results of *in vitro* digestibility methods, including protein digestibility, with the conventional digestion trial to determine if *in vitro* methods can be used as effectively to detect differences in digestibility.

2. To study the rate of maturation of forage varieties to determine if varietal differences are maintained throughout the growing season.

3. To study the chemical composition and *in vitro* digestibility of the leaf, sheath, stem and seedhead fractions of grasses to determine their usefulness in plant breeding and selection programs.

REVIEW OF LITERATURE

Proximate Analysis and Digestibility Trials

Numerous methods of feedstuffs evaluation have evolved in the past century of progress in animal nutrition; however, the most generally used scheme for describing feedstuffs was developed before this time. The proximate analysis scheme was devised by Henneberg and Stohmann at the Weende Experiment Station in Germany as a result of their investigations from 1858 to 1863 (Hansen et al., 1958). According to this scheme of analysis a feedstuff is partitioned into six fractions, water, ether extract, crude fiber, nitrogen free extract, crude protein and ash. Five of these fractions are determined chemically, while the sixth, nitrogen free extract, is determined by difference. This plan of analysis groups together a variety of substances in terms of some of their common chemical characteristics. Each of the components, except water, represents a combination of nutrients some of which are of little nutritional value to the animal.

Shortcomings of the proximate analysis system such as the inclusion of lignin in the nitrogen free extract fraction rather than in crude fiber and the calculation of nitrogen free extract by difference have been discussed by many authors. The reader is referred to a more recent review by Van Soest (1967) for a discussion of the limitations of the proximate analysis system. Regardless of the shortcomings of the proximate analysis system, with proper interpretation of the figures for the carbohydrate fractions it gives one adequate information about the class of animal the feed would be most suitable

for. The application of the proximate analysis scheme to forage evaluation has greatly increased our knowledge of forage utilization in feeding practice.

Chemical analysis of feeds attempts to predict the nutritive value of feeds; however, this does not provide information as to what components of the feed are actually available to the animal. Actual digestibility of the feed must be known since undigested nutrients do not enter into body metabolism.

A digestion trial involves a record of the nutrients consumed and the relative fecal losses. It is essential that the feces collected represent, quantitatively, the undigested residue of the measured amount of food consumed (Maynard and Loosli, 1962). In carrying out a digestion trial the animals are placed on the test ration for a number of days before collection of feces is initiated. This is termed the preliminary period and may vary in length depending on the ration being tested. The effect of length of preliminary periods has been studied by Hall and Woolfolk (1952), Nicholson et al. (1956) and Lloyd et al. (1956). These researchers concluded that there is little justification for extending the preliminary period beyond 10 days when only minor changes are involved in the test rations. They did conclude that longer preliminary periods are needed when hay to grain ratios vary widely. The optimum preliminary period for such trials lies between 16 and 30 days. Hall and Woolfolk (1952) extended the preliminary feeding period to 60 days and found rhythmic fluctuations in the digestibility of all components of the ration.

They mentioned that because of these fluctuations it seems highly improbable that constant and maximum digestibility of the nutrients studied is biologically normal.

Total collection procedures are usually employed as a standard procedure when exacting data are required. This involves an accurate accounting of all feed consumed and of all feces excreted during the test period and necessitates the use of metabolism stalls or fecal collection bags. Precautions must also be taken to avoid contamination of feces with urine.

The digestibility of roughages may present problems in relation to practical feeding conditions since refusal of animals to consume portions of roughages is often encountered. Several procedures have been proposed to overcome this problem. The first method is to feed at restricted levels to encourage animals to consume the feed allowance, and the second is the preparation of feeds in such a manner that selection by the animal is eliminated (Maynard and Loosli, 1962). A common procedure is to collect the refused portion, dry, weigh and analyze it. This quantity is then added to the nutrients excreted in the feces and the sum subtracted from the nutrients fed (Lindahl, 1963). All of the above procedures measure the digestibility of the roughage as consumed during the trial but may give misleading data about the nutritive value of a feed when applied to practical feeding conditions (Lindahl, 1963). This may not be a problem with high quality forages which are readily consumed, but total digestible

nutrients calculated on the "as consumed basis" for low quality, stemmy forages overestimates their value.

Indirect methods of determining digestibility have also gained wide acceptance. Studies by Kane et al. (1953) and Elam et al. (1962) using indicators such as chromic oxide, chromagens and lignin yielded good comparisons with total fecal collection methods. In the above studies coefficients of digestibility determined from single day collections of feces by the chromic oxide ratio method agreed well with those obtained by the total collection methods. Values calculated from the lignin ratio method were significantly lower. The indicator methods of determining digestibility are of particular value in pasture and range forage evaluation studies.

As with all methods of forage evaluation the digestion trial has also been a subject of controversy as to its accuracy. Schneider and Lucas (1950) reported on the magnitude of certain sources of variability associated with digestibility trials. They found that the largest errors were associated with the ether extract fraction followed by crude protein and crude fiber, while the variability of total digestible nutrients was smallest. They concluded that accurate average digestibility data cannot be obtained by one worker or a few workers each conducting a large number of trials. They suggested cooperative studies for attaining this goal. Collaborative studies reported by Donefer (1966) and Barnes (1968) indicate that variability among experiment stations in determining the digestibility of a common feed may be significant. Both authors mentioned that the variation in

determination of chemical constituents and their digestibility emphasizes the importance of sampling technique and preparation of forages for analysis. The study reported by Barnes (1968) indicated that the magnitude of variability among sheep within stations was greater than that among stations for dry matter digestibility measured during the restricted intake phase. In spite of the fact that a high within feed variability in digestibility is very common, Schneider et al. (1950) have shown that the application of average digestion coefficients in addition to chemical composition data resulted in appreciable gains in precision when predicting nutritive value.

Total digestible nutrients (TDN) are based on data obtained in conventional digestion trials. Calculation of TDN is made by multiplying the amount of crude protein, crude fiber, nitrogen free extract and ether extract by the digestion coefficients for the respective constituents and assigning an energy value of one to digestible protein, fiber and nitrogen free extract and 2.25 to digestible ether extract (Lindahl, 1963).

Total digestible nutrients are the easiest measures of food energy to determine. Published average digestion coefficients make possible the calculation of a given supply of feed from its specific nutrient content. The limitation of TDN as a measure of food energy is that the errors associated with the proximate analysis system of feed analysis are inherent in the calculation of TDN. In addition it does not take account of the other important losses, such as the combustible gases in the case of Herbivora and, most important, the

heat loss (Maynard and Loosli, 1962). These losses are considerably larger for roughages than for concentrates and it is for these reasons that TDN overestimates the value of roughages. Lindahl (1963) mentions that although TDN has a number of serious limitations it is easy to determine, values are available for a large number of different feedstuffs, the values serve as the basis for the feeding standards currently employed in the United States, and undoubtedly TDN will be the basic system of energy evaluation during the next few years.

The use of digestible energy as a measure of food energy eliminates the errors associated with the proximate analysis system and consequently is considered to be a more reliable measure of food energy than TDN. The calculation of digestible energy involves the determination of the gross caloric content of the feed consumed and the feces excreted. Although superior to TDN as a measure of food energy, digestible energy does not account for any additional losses other than those associated with digestion.

It is ironic that at the time of Lindahl's writing the work of Lofgreen (1963) to develop net energy requirements for maintenance and net energy requirements for production came into limited use. Although this system also has some limitations, it accounts for more of the losses which occur in the digestion and utilization of forages. According to Lofgreen and Garrett (1968) previous net energy systems were not recommended for use under maintenance conditions because of their tendency to underevaluate roughages in relation to concentrates for maintenance. The proposed system appears to be applicable to

maintenance conditions as well as production. However, the above workers do not take into consideration that this system is largely based on values calculated from existing TDN data. In addition they fail to mention that this system assumes that animals will be kept in a "zone of thermal neutrality." For this reason rations based on TDN may be more appropriate for colder climates. The California system, although in its infancy, will undoubtedly become more widely used in feedstuff evaluation in coming years.

Cellulose and Lignin

The insoluble residue that remains after digestion with dilute alkali consists largely of cellulose as a polymer of beta-D-glucose. Since the early recognition that crude fiber and nitrogen free extract did not represent pure chemical entities, numerous attempts have been made to classify the carbohydrate fraction of feedstuffs by more precise methods. This eventually led to the separation of carbohydrates into such component fractions as cellulose, hemicellulose, holocellulose and lignin. Cellulose and lignin are of primary interest in this discussion.

One of the early schemes of analysis proposed to avoid the limitations of crude fiber and nitrogen free extract as measures of forage nutritive value was that of Crampton and Maynard (1938). Based on studies of previous procedures, methods were developed for the determination of cellulose and lignin directly while "other carbohydrates" were estimated by difference. Their data indicated that, at least for Herbivora, a partition of the carbohydrate portion of a

feed into lignin, cellulose and other carbohydrates may have more biological significance and hence be of greater usefulness in predicting feeding values. Their method of cellulose analysis has been quite widely accepted in contrast to the lignin procedure. A modification of the above scheme was proposed by Crampton and Whiting (1943) in which cellulose and other carbohydrates were determined and lignin calculated by difference. This method of analysis, although believed to more nearly approximate biologically related units, has not been widely accepted. Matrone et al. (1946) modified the Norman-Jenkins method to shorten the time and increase the efficiency of the operation with the result that this method has been widely used. These authors mention that crude fiber is an approximate measure of true cellulose and that with legumes the agreement between crude fiber and cellulose is relatively close, while with grasses the cellulose values are greater by approximately 30%, indicating that grasses contain more xylan than legumes. Hansen et al. (1958) in their review of carbohydrates pointed out that, since ruminants seem to utilize cellulose and pentosans with equal gross efficiency, a method which groups these items as a "biological unit" has merit in terms of probable usefulness to animals.

It appears that cellulose is a better index of feeding value than crude fiber at least for forages. The crude fiber value becomes less reliable as an index of the poorly digested carbohydrate fraction of grass forages with advancing maturity.

Carbohydrate entities such as hemicellulose and holocellulose have also been the subject of considerable investigation; they are discussed briefly. Holocellulose includes both hemicellulose and cellulose and therefore represents nearly all of the carbohydrate present in forages other than the soluble carbohydrate. A study by Ely and Moore (1956) indicated that protein and lignin are minor contaminants of holocellulose prepared from materials low in protein. However, when preparations are made from high protein materials, the apparent lignin and protein content of holocellulose increases.

Studies of the hemicelluloses of forage plants by Sullivan (1966) revealed that there may be a closer association between hemicellulose and lignin than between cellulose and lignin. This was illustrated by the fact that hemicelluloses were not water soluble but became so when delignified as in the preparation of holocellulose. The hemicelluloses of grasses were more digestible than legumes and in both types of forage the digestibility of the carbohydrates was adversely affected by the quantity of lignin, and a higher ratio of hemicellulose to cellulose was associated with higher digestibility.

Although lignin is not a carbohydrate, it is included in the carbohydrate fraction by association. The presence of lignin in forage material decreases digestibility and utilization of the feed in question and this depression becomes increasingly greater with advancing maturity. The specific effects of lignin on forage quality will be discussed in the section dealing with changes in components of plants with advancing maturity. The discussion here is limited to the

development of analytical methods. A considerable amount of the early work involving lignin analysis in forages was done by Norman and Jenkins (1934). They showed that pentosans may be hydrolyzed and converted to furfuraldehyde slowly in the presence of 72% sulphuric acid and that furfuraldehyde may combine with lignin, resulting in high apparent lignin values. Under appropriate conditions, 2 hr. at 20° C., this effect was minimal, and this still remains the basis for 72% sulphuric acid treatment.

The procedure developed by Crampton and Maynard (1938) contained a pretreatment period with pepsin for 12 hr. to reduce the effect of protein on the lignin values. Earlier Norman and Jenkins (1934) had shown that lignin isolated from plant materials of high protein content, contained more nitrogen than lignin isolated from materials low in protein. The study of several lignin analysis methods by Thomas and Armstrong (1949) revealed that the Crampton and Maynard method gave considerably higher yields of lignin than the Norman and Jenkins procedure. The authors attributed this to the alcohol-benzene extraction which did not completely remove waxes and other substances which would interfere with the penetration of 72% sulphuric acid and thereby enhance yields. Also, the addition of the formaldehyde used is capable of increasing the weight of lignin. The method of Ellis et al. (1946) has been widely accepted. Basically it is the method of Norman and Jenkins with the addition of an acid-pepsin digestion prior to the 5% sulphuric acid treatment. Other factors which may affect the lignin values in addition to nitrogen

content of the material are alcohol-benzene extraction time and drying temperature of the samples. Results of Thomas and Armstrong (1949) indicated that a 4 hr. extraction with alcohol-benzene was insufficient for complete extraction, while a 30 hr. period was longer than necessary. It was also shown that oven drying of feces at a temperature of 105° C. resulted in higher lignin values than similar samples dried at 60° C., although the difference was not as marked as with feed samples. Ellis et al. (1946) and Gaillard (1962) showed that high drying temperatures increased the lignin content of young immature tissue more than in plant tissue of advanced maturity.

It should be pointed out that the methods discussed are presently used very little. This is in part due to the time consuming nature of the methods as well as the development of new analysis procedures by Van Soest (1963a). The development and application of this method is discussed in the following section.

Development of Laboratory Methods of Forage Evaluation

Carbohydrate and Lignin Analysis. The present use of crude fiber in forage analysis is based on the assumption that crude fiber is related to quality. While this is generally true, the precision of the method decreases when plants become mature. Crude fiber may be unsatisfactory because of its variable composition and digestibility (Norman, 1935). It is sometimes more digestible than the nitrogen free extract (NFE), which is supposed to represent the highly digestible carbohydrates. The low digestibility of NFE results partly from extraction of indigestible lignin and partially from digestible

hemicellulose in the fiber determination. With these limitations in mind, Van Soest (1963a) initiated the development of a new system of analysis for nonnutritive residues of feeds. Although this system was designed primarily for use with roughages, it is also applicable to less fibrous feeds. The method of analysis for fiber by Van Soest (1963a) began with the examination of several possible detergents. Two combinations seemed to be promising with regard to preparation of plant fiber of low nitrogen content, sodium lauryl sulfate in neutral or slightly alkaline solutions and cetyltrimethylammonium bromide in strongly acid solution. These two detergents and media yield two basically different types of fiber. The fiber prepared from neutral detergent solution represents cell wall constituents (CWC) in essentially undegraded form. The fiber prepared by acid digestion gave a considerably smaller yield and probably represents the more indigestible portion of the fiber, at least as far as ruminants are concerned. It was pointed out that the latter residue would be a more suitable starting material for lignin analysis, since a considerable portion of the hemicellulose would have been removed.

Further work by Van Soest (1963b) led to the present terminology of acid-detergent fiber (ADF) and acid-detergent lignin (ADL). Basically the ADF analysis is an extraction of plant material in 1 N sulphuric acid containing 20 gm. of cetyltrimethylammonium bromide per liter for 1 hr. The residue obtained after filtration and drying is termed ADF. The determination of lignin from ADF is accomplished by a 2 hr. extraction with 72% sulphuric acid at room temperature after

which the material is washed free of acid, dried, weighed and ashed and ADL determined as the loss on ignition.

In applying the ADF and ADL analyses to forages of known digestibility, Van Soest (1963b) found that ADF ranged from 25 to 54% and ADL ranged from 2.0 to 11.5%. The composition of the ADF fraction did not appear constant for carbon, nitrogen and ash. The variations in carbon and ash content may have been related to the maturity of the forage. Correlations between digestibility and ADF were higher than with crude fiber. When correlations were calculated between lignin and digestibility for legumes and grasses separately, they were higher than when combined. Values for grasses and legumes were -.88 and -.93, respectively. Although lignin decreases the digestibility of carbohydrates, the exact linkage or association is not known. Studies by Bolker (1963), Van Soest (1964) and Sullivan (1966) indicate that the point of attachment may be mainly on the hemicellulose portion of holocellulose. The study of lignin in wood and sulphite pulps by Bolker (1963) suggested that the linkage is an acetal or hemi-acetal bond and on cleavage liberates a ketone group in the lignin.

The fiber analysis methods of Van Soest have been successful because the detergents used allow for removal of protein and other plant substances without disruption of the fibrous components. In addition the ADF fraction includes lignin which is partially lost in the crude fiber analysis. The removal of protein is more complete in the ADL procedure than in previous methods of lignin analysis

(Van Soest, 1963b); consequently, the values obtained are more representative of "true lignin."

Differences in chemical composition between crude fiber and acid-detergent fiber have been studied by Kim et al. (1967) and Colburn and Evans (1967). The study of Kim et al. (1967) indicated that the ADF procedure invariably yielded higher results. This difference varied directly with lignin content of the original material and was most pronounced in fecal material. The ADF contained more lignin but less pentosans and cellulose than crude fiber. The crude fiber fraction lost 60 to 84% of original lignin and 80 to 86% of original pentosans. In the study of Colburn and Evans (1967) cellulose and lignin accounted for about 90% of the ADF for grasses and about 94% for alfalfa-grass mixtures. They also stated that lignin was completely recovered in the ADF fraction and in this respect ADF would be considered more representative of the indigestible portion of a feed.

The ADL method of analysis is also sensitive to the effects of heating as shown by Van Soest (1965a). It was shown that heat drying of forages at temperatures above 50° C. caused significant increases in yield of lignin and fiber. The results showed that heating air-dry forages produced smaller increases in the ADL, insoluble protein and apparent lignin than that obtained in drying wet forage at 100° C. for 20 hr. The harmful effect of high temperatures appeared to take place in the first few hours of the drying process.

In addition to fiber and lignin analysis methods, Van Soest and Marcus (1964) developed a procedure for the determination of cell

wall constituents (CWC) which was later modified by Van Soest and Wine (1967). The procedure involves the extraction of plant material in a buffered detergent solution for one hour. The residue obtained contains lignin, hemicellulose, cellulose and some wall-bound protein. It was shown that the digestibility of the insoluble residue appears to be much less than that of the soluble portion. Cell wall constituent values were more closely related to voluntary intake than dry matter digestibility, although there was no significant relationship between CWC and voluntary intake in forages with a CWC content of less than 60% of the dry matter. Cell wall constituents which represent the total fibrous fraction of the forage limit intake when they increase to more than 60% of the dry matter (Van Soest, 1965b; Van Soest and Wine, 1967). Cell wall data fit intake data much better than cellulose, lignin or other plant fractions, none of which measure all of the fibrous constituents comprising the cell wall. Van Soest (1965b) also mentioned that the CWC of grasses may be greater than those of legumes, resulting in about equal dry matter digestibility. The voluntary intake is usually lower, especially in the more mature grasses that are highest in quantity and lignification of CWC. In the case of legumes, the fibrous mass ingested is not large enough to inhibit intake, and legumes as a class are characterized by optimum intakes.

The application of the methods of Van Soest for the prediction of digestibility is discussed by Van Soest (1965c). The equation was developed by assuming that the principal factors governing

digestibility (DDM) of feeds are the proportion of completely available cell contents (S) and the concentration of lignin (L) in ADF, which controls the digestibility of CWC (W). The equation was DDM = 0.985 + W(147.3 - 78.9 log L) - 12.9. Correlation of estimated digestibility with *in vivo* digestibility was 0.96.

In summarizing the development of a comprehensive system of feed analysis and its application to forages, Van Soest (1966) stated that forage dry matter can be divided into two fractions on the basis of nutritional availability. The first fraction corresponds to the cellular contents and is composed of lipids, soluble carbohydrates, most protein and other water soluble matter. This fraction is essentially available, but its digestibility appears incomplete because of the excretion of fecal noncell wall material. The second fraction corresponds to the plant cell wall, the availability of which is controlled by structural features that link cellulose, hemicellulose and lignin together. The plant cell wall corresponds to what can be nutritionally defined as the total fiber fraction. Van Soest (1967) also mentioned that it is the dual nutritive character of plant dry matter which contradicts the use of single factors to predict dry matter digestibility.

The methods of fiber analysis developed by Van Soest and co-workers have gained wide acceptance since their introduction. The procedures overcome the objections of previous methods in that they are more accurate entities and the ease of performing the analyses is greatly facilitated. Acid-detergent fiber and lignin are highly

inversely correlated with nutritive value and can be successfully used to predict the same. Cell wall constituents are more indicative of voluntary intake and therefore when combined with ADF and ADL in feeding practice should provide exceptionally good estimates of forage nutritive value.

Although the methods have not been heavily scrutinized, their widespread use indicates acceptance. Certainly, they are a major improvement when compared to crude fiber and lignin analyses procedures of the past.

Chemical Solubility Methods. The use of cellulose solubility as an estimate of cellulose digestibility was initiated by Dehority and Johnson (1961a). Their preliminary study involved a two-hour extraction with 30 ml. of cupriethylene diamine (CED) followed by two washings with CED and analysis for residual cellulose. Results of this study indicated that the percent of forage cellulose dissolved was linear and highly correlated with the percent of cellulose digested in the *in vitro* rumen fermentation. Comparisons of the solubility data with *in vivo* cellulose digestion for 12 grass samples and effective nutritive value index of 8 grass samples showed marked linear associations; however, attempts to correlate cellulose solubility of alfalfa with *in vitro* and *in vivo* data were unsuccessful. A subsequent study by Dehority and Johnson (1961b) showed that the amount of cellulose which could be dissolved from timothy samples decreased with advancing maturity of the plant. In the early studies with CED, a large day to day variation was observed in the percent cellulose dissolved from a particular forage.

A study by Dehority and Johnson (1963) was concerned with increasing the precision of the method and determining its relationship to *in vitro* and *in vivo* measurements of digestibility. Various steps in the procedure were modified, so that a standard method with fairly high precision resulted. The values obtained by this method were correlated with *in vitro* cellulose digestion, *in vivo* cellulose, dry matter and energy digestion, nutritive value index and relative intake. The correlations with all criteria except relative intake were highly significant.

In view of the fact that the CED method was not applicable to legumes, the method was modified by Dehority and Johnson (1964). The insolubility of cellulose of legumes was thought to be due to a masking effect by some substance other than lignin. From preliminary data, it was concluded that extraction with 1.0 N sulphuric acid yielded maximum cellulose solubility. The improved CED method gave a correlation of 0.83 with *in vitro* cellulose digestion, although the correlation with nutritive value index was very low. Since the amount of cellulose soluble in CED was not highly correlated with relative intake for grasses, legumes or mixed forages, the authors determined the dry matter soluble in 2.0 N sulphuric acid much in the same manner as the CED procedure. Correlation of dry matter solubility (DMS) with relative intake resulted in a coefficient of 0.90. When the data from all types of forages were combined and correlated against CED and DMS, methods as well as the product (CED x DMS) of the two methods, the best correlations obtained were as follows: dry matter digestibility

(CED x DMS), 0.87; energy digestibility (CED x DMS), 0.88; NVI (DMS), 0.83 and relative intake (DMS), 0.78.

A study by Johnson *et al.* (1964) comparing several *in vitro* methods and solubility procedures indicated that correlation coefficients obtained with CED were much less consistent than others, while DMS was highly correlated with dry matter digestibility for alfalfa but not for the other forages. *In vitro* cellulose digestibility measurements were more highly correlated with *in vivo* measurements for grasses, while CED, DMS or both were better for alfalfa and mixed forages. The authors also indicated that between run variability was greatest for the *in vitro* cellulose digestibility method. Similar results were reported by Oh *et al.* (1966). Johnson and Dehority (1968) in a comparison of the predictive value of several methods found that relative intake and nutritive value index were predicted most accurately by *in vitro* cellulose digestibility x DMS or CED x DMS. Dry matter and energy digestibility were most accurately predicted by a two stage *in vitro* digestion procedure.

The high correlations obtained with the chemical solubility methods indicate that they can be of use in estimating forage quality. Although the correlations obtained are not as high as those obtained with *in vitro* rumen fermentation techniques they are highly significant. Possibly the greatest advantage of the chemical solubility techniques is that they can be employed in any laboratory without the equipment and facilities necessary for *in vitro* fermentation techniques. Also, variability between runs is eliminated or greatly reduced, a factor

often encountered when using rumen inoculum in *in vitro* fermentation techniques.

In Vitro Cellulose Digestibility. Early attempts at developing *in vitro* systems were largely based on incubating substrates with strained rumen fluid and measuring activity by several criteria. The publication of the mineral composition of sheep saliva by McDougall (1949) provided great impetus to the investigation of *in vitro* techniques. Nearly every *in vitro* system developed since that time uses a mineral mixture based on "McDougalls solution" or on a mixture originally based on it and later modified.

A considerable portion of the early development of the *in vitro* system involved the determination of nutrient requirements, inoculum source, length of fermentation and the effects of numerous other factors known to affect digestibility. Studies by Burroughs *et al.* (1950a), Burroughs *et al.* (1950b), Arias *et al.* (1951), Burroughs *et al.* (1951), Bentley *et al.* (1954), Cheng *et al.* (1955), Huthanen and Elliot (1956) and MacLeod and Murray (1956) were all concerned with the effect of various minerals, vitamins and other nutrients on the digestibility of cellulose *in vitro*. These studies indicated that numerous minerals and vitamins were stimulatory to *in vitro* cellulose digestibility (IVCD) and also that some elements were inhibitory. It was also through these initial studies that it was shown that whole rumen fluid, in which feed particles had been removed, provided most of these nutrients and eliminated the need for providing them in the media. In addition,

whole rumen fluid provided undetermined factors which enhanced microbial activity.

The source of rumen inoculum and the method of its preparation prior to use have been investigated by Johnson et al. (1958), LeFevre and Kamstra (1960), Church and Peterson (1960), Donefer et al. (1960), Shelton and Reid (1960), Donefer et al. (1961), Bowden and Church (1962a) and Wallace et al. (1965). The results of these studies showed that rumen fluid obtained from cattle and sheep maintained on the same ration did not significantly affect IVCD. In addition, it was shown that the ration fed the donor animal may affect the level of activity of the rumen inoculum although this variation was not significant with the exception of poor quality prairie hay (Bezeau, 1965).

The methods of preparation of rumen inoculum include strained rumen fluid, phosphate buffer extracts, rumen fluid from pressed pulp of ingesta and resuspended microorganisms. These methods of preparation have been studied by Johnson et al. (1958), Donefer et al. (1960), Donefer et al. (1962) and Wallace et al. (1965). Generally methods which involve buffer extracts or resuspended microorganisms yield an inoculum of lower cellulolytic activity and consequently IVCD values are decreased when compared to strained rumen fluid. This is attributed to a removal of essential nutrients present in the rumen fluid and a failure to provide these in the fermentation media. Johnson et al. (1958) also showed that aeration decreased activity steadily, a process which may occur unintentionally during the preparation of

resuspended inoculum. Shelton and Reid (1960) in their discussion of *in vitro* methods indicate that there is little evidence to suggest that *in vivo-in vitro* relationships are improved by the use of washed cell suspensions.

It would appear that the most important factor relative to rumen inoculum is that the donor animal should be maintained on the same ration for all *in vitro* samples to be compared with one another. Also, if possible, the animal should be maintained on a ration similar to or closely related to the material being investigated.

The length of *in vitro* fermentation periods was studied extensively during the early development of the IVCD procedure. The study of LeFevre and Kamstra (1960) showed that a 24 hr. IVCD value underestimated *in vivo* digestibility while a 48 hr. fermentation most nearly simulated the *in vivo* data. Studies by Reid *et al.* (1960), Barnes *et al.* (1964) and Baumgardt and Oh (1964) indicated that correlations between IVCD and *in vivo* criteria are highest when 24 or 48 hr. fermentations are used. These workers also indicated that variability between duplicate samples and individual runs decreased as the length of fermentation period increased.

Relatively short-term fermentations have been used by Donefer *et al.* (1962) and Karn *et al.* (1967) for the prediction of nutritive value index and cellulose and dry matter digestibility, respectively. The results of these studies indicate that short-term rate studies may be more useful in predicting relative intake and nutritive value index than longer fermentation periods.

In addition to the above mentioned variations and modification of techniques, another important change in the IVCD procedure is the reduction in the size of sample which has taken place throughout the years. In the early stages of development samples as large as 20 gm. were used. This was gradually reduced because of the limited number of samples which could be included in one fermentation. Along with the decrease in sample size was the use of smaller fermentation vessels. Presently the 100 ml. test tube is the most common vessel in use.

The use of carbon dioxide to maintain anaerobiosis in the fermentation vessel is discussed by Johnson (1966) in his review of *in vitro* procedures. In early methods this was accomplished by continuous gassing with carbon dioxide by the use of a tube extending near the bottom of the fermentation vessel. More recently the use of a Bunsen valve has gained wide acceptance. This is usually no more than a rubber policeman placed over an outlet tube. A small slit is made to allow gas to escape but prevent air from entering. This system depends on microbial metabolic production of CO_2 during the fermentation period. The use of the Bunsen valve also facilitated the inclusion of a large number of samples within a fermentation run since the need for tubing directly to each vessel was eliminated.

The *in vitro* cellulose digestibility procedure has been widely accepted for evaluation of small quantities of forage and for the study of factors which influence cellulose digestibility. Studies by Salsbury *et al.* (1955) and Baker *et al.* (1959) on the rates of digestion of different celluloses indicated that all types of cellulose

are not digested at the same rate. The data showed that wood cellulose and cotton linters were rapidly attacked for a short period of time immediately after incubation but this decreased sharply after 8 hr. It was also shown that forage cellulose gave a smooth curve showing a uniform decrease in rate of digestibility. Kamstra and Thurston (1961) used IVCD to determine the digestibility of holocellulose, cellulose within holocellulose and intact plant samples. They found that the holocellulose was more digestible than either of the other fractions.

The IVCD procedure has also proven to be an invaluable aid in studying the effects of lignification on cellulose utilization. The study of Kamstra et al. (1958) showed that when the whole plant was digested IVCD values were lower than for samples isolated from the plant either as a holocellulose or as cellulose. They suggested that lignin functioned as an encrusting agent preventing digestive juices from acting on the cellulose. Dehority et al. (1962) studied the effect of lignification on the IVCD of hemicellulose and pectin. It was found that the rate of IVCD for both hemicellulose and pectin decreased with advancing maturity. When the samples were subjected to ball milling and then analyzed, digestibility was increased with the increase being largest for the most mature forages, again suggesting an encrustation of lignin. These data are in agreement with that of Dehority and Johnson (1961b). Tomlin et al. (1965) found that lignin content was negatively correlated with IVCD at the 12 hr. period for grasses and legumes, although the regression equations for the two groups were significantly different. Lignification was linearly

related to IVCD as the grasses matured; however, this relationship did not exist for alfalfa.

Numerous investigations have been made concerning the correlation of IVCD values with *in vivo* criteria and the predictive value of IVCD. Baumgardt *et al.* (1958) and Hershberger *et al.* (1959) obtained correlations of 0.90 and 0.97, respectively, between IVCD and *in vivo* digestible cellulose values. These workers also found IVCD to be significantly correlated with *in vivo* energy digestibility. The values were 0.80 and 0.92, respectively. These correlations are comparable to those reported by other workers. Further work by Baumgardt *et al.* (1962a, 1962b) showed that IVCD was also significantly correlated with total digestible nutrients and digestion coefficients for dry matter, organic matter and energy. These studies indicated that a 24 hr. fermentation period for IVCD gave a higher correlation with digestible energy than a 48 hr. fermentation.

Studies by Johnson *et al.* (1962) and Chalupa and Lee (1962) involving the use of IVCD to predict nutritive value of forages indicated that a 12 or 18 hr. fermentation period compared most closely with the nutritive value index, especially when alfalfa was included. When longer fermentation periods were considered, the correlations with *in vivo* measurements were quite high for grasses alone but much lower when alfalfa data were included in the analyses.

The utilization of IVCD as a predictor of forage value has been a valuable asset in the development of more comprehensive knowledge concerning forage quality and utilization. However, the limitation of

the method may be that only one component of the forage is being evaluated even though numerous other factors may affect the utilization of this component.

In Vitro Dry Matter Digestibility. As the IVCD technique was refined and sample size was being reduced to one-half gram, difficulties arose when the residual matter was analyzed for cellulose. The residues were quite small and repeatability was low. In addition, it was also shown that only one component was being estimated and this did not appear to give acceptable results when legumes were considered. A study by Tilley et al. (1960) showed that there was close agreement between in vitro and in vivo dry matter digestibility with feeds of relatively low (below 60%) digestibility and of low protein content. Feeds with higher digestibilities and higher protein content had in vitro digestibilities as much as 10% lower than corresponding in vivo figures. These authors suggested that although most of the digestion of ruminants takes place in the rumen of the animal, further digestion, especially of protein, occurs lower down the digestive tract. This digestion is not simulated with in vitro rumen liquor digestion. It was on this basis that these workers proposed a secondary digestion with proteolytic enzyme "pepsin." As a result, the use of the pepsin stage reduced the standard error of the estimate and also altered the slope of the regression line. The pepsin digestion increased all values closer to those obtained in vivo with the largest increases occurring with those samples having the highest crude protein content. It should

also be mentioned that *in vitro* dry matter digestibility (IVDMD) procedures eliminate the need for cellulose analysis of the sample and residue. This is a major factor for the increasing use of IVDMD since as many as 300 samples are digested simultaneously.

In a more complete description of their two stage IVDMD procedure Tilley and Terry (1963) mentioned that some variation in digestive efficiency between experiments seems to be unavoidable and wherever possible similar forages should be compared within the same experiment. Their study also indicated that it is essential to maintain anaerobic conditions throughout the first stage of the experiment with thorough gassing of the solutions and tubes with carbon dioxide and attention to the condition of the gas release valves being most important. The effect of drying temperature and grinding on IVDMD was also investigated. These workers concluded that drying for one or two days at 100° C. was safe and that samples need only be ground fine enough to insure good sampling of the small weights of forage used. The results of this study with 148 forages of known *in vivo* digestibility showed that the correlation between IVDMD values and *in vivo* dry matter digestibility was high.

Barnes (1966) in a study designed to evaluate the worth of various solubility and *in vitro* rumen fermentation procedures for estimating *in vivo* dry matter digestibility found that a 48 hr. fermentation followed by a 24 hr. pepsin digestion gave the highest relationship with *in vivo* dry matter digestibility ($r = 0.97$). The

technique was believed adequate for the simultaneous evaluation of both grasses and legumes.

The IVDMD procedure has also been used extensively in the evaluation of plant fractions by Pritchard *et al.* (1963) and Mowat *et al.* (1965b, 1965c). In studies of this nature where sample material is limited to several grams, IVDMD has a definite advantage in that less material is required for the analysis in comparison to IVCD. A procedure of Clark and Mott (1960) utilizing a type of dialysis bag for *in vitro* dry matter digestibility yielded values significantly correlated with *in vivo* data. A study by Bowden and Church (1962b) also indicated that correlations between IVDMD and *in vivo* digestibility were highly significant. Both of the above mentioned procedures did not utilize a pepsin digestion. The relationship between IVDMD and *in vivo* DMD as studied by O'Shea and Wilson (1965) and Wilkins (1966) revealed highly significant correlations between the two methods.

The comparison of several laboratory techniques for predicting *in vivo* DMD by Oh *et al.* (1966) and Johnson and Dehority (1968) showed that *in vivo* DMD was most accurately predicted by the two stage IVDMD technique. Oh *et al.* (1966) suggested the possibility of using one method and a single regression equation for all species. The equation developed was $y = 16.7 + 0.74x$, where y is equal to estimated *in vivo* DMD and x is IVDMD. The application of bomb calorimetry to undigested residues to obtain *in vitro* digestible energy values did not improve the *in vitro*-*in vivo* correlations.

It should be pointed out that the IVDMD method is not a standardized method used by many laboratories, although it would appear so from the literature. As is often the case, experiments are reported as using the procedure of Tilley and Terry (1963); however, minor changes may have been made in the procedure which may affect the results obtained. It is the author's opinion that any modification of the procedure should be clarified to facilitate comparison of data among laboratories. The report of a collaborative *in vitro* rumen fermentation study by Barnes (1967) showed that the mean *in vitro* digestibility for common forages by individual laboratories after a 24 hr. fermentation period ranged from 40.0 to 63.9% for cellulose and 38.7 to 53.3% for dry matter. The study also indicated that variability among laboratories was greater than among runs within laboratories which was greater than between duplicate determinations within runs. The precision of the *in vitro* methods did not differ greatly among laboratories, except for individual cases, and the precision of the techniques was greatest at the longer fermentation periods.

The variability present with *in vitro* techniques and also with *in vivo* criteria as mentioned previously point out that the prediction equations developed by one laboratory should not be used by another laboratory, even though identical procedures may have been used.

Tilley and Terry (1963) pointed out that *in vivo* digestibility is not a constant characteristic of a forage and this, in itself, sets a limit to the accuracy with which *in vivo* digestibility can be predicted from any analysis of forage. They also mentioned that for

this reason it would seem preferable to report *in vitro* digestibilities, determined under specified conditions, rather than to attempt to predict *in vivo* digestibility.

The *in vitro* techniques have proven extremely useful in the study of plant fractions where only small samples are available. Also, the use of these techniques by plant breeders in the selection of desirable genetic material early in the propagation process could be of great benefit. The screening of a large number of samples is possible with a minimum amount of time required. Although the *in vitro* methods provide relative information as to forage quality, they should not be used as the ultimate criteria since they do not measure animal acceptability. This factor must be considered since forage quality depends upon the rate at which it is consumed and its energy value per unit of weight.

Effects of Advancing Maturity on Important Components and Digestibility of Intact Plants

Changes in the relative amounts of fiber, protein and lignin in plants associated with advancing maturity and the effects of these changes on digestibility of plants are of particular interest to the plant breeder. Varieties of forage or breeding material may be similar in composition and digestibility when harvested at the recommended stage of growth. However, the rate of maturation may not be similar causing some varieties to be less desirable than others when harvested at a date later than recommended. A knowledge of the rate of maturation would be

useful to the plant breeder in selecting plants which remain high in digestibility at advanced stages of maturity. Changes in fibrous components, protein and cellulose and dry matter digestibility are discussed.

Although the proximate analysis system of feedstuff classification had been in use for some 40 years, the development of a cellulose and lignin analysis procedure by Crampton and Maynard (1938) greatly facilitated the investigation of the effects of advancing maturity on components of forage plants. Early studies by Patton and Gieseker (1942) and Patton (1943) using the procedure of Crampton and Maynard showed that lignin content increased with advancing maturity of six Montana grasses and that the lignin content closely paralleled cellulose content. These workers indicated that considerable species variation existed, with grasses in the more arid region reaching their maximum lignin values earlier in the season. An interesting study by Pigden (1953) showed that in native grasses lignification was confined to the vascular bundles while introduced species exhibited lignification in the surrounding tissue as well. The lignin in this case was the most important factor contributing to the "curing" property of native grasses. The position and extent of lignified tissues appeared to be more important than the quantity present. Studies by Phillips et al. (1954), Kivimae (1960), Lloyd et al. (1961) and Gaillard (1962) all showed that during maturation the percentage crude fiber, cellulose, and lignin in forage plants increased. The study of Lloyd et al. (1961) showed that crude fiber and cellulose content of plants sampled from

the early bloom to past bloom stage increased with later maturity but were not of great magnitude. In contrast, lignin content doubled even though the increase in lignin was slower after the seed began to form. Phillips et al. (1954) and Kivimae (1960) both indicated that lignin was preferred over crude fiber as an indicator of forage digestibility.

Denium (1966) reported that in addition to advancing maturity such climatological factors as light intensity, temperature and water supply during the growth period considerably influence the chemical composition of forage plants. These findings were similar to those reported by Bowman and Law (1964). These studies indicate that the chemical composition may largely be determined by the climate in which the plant is grown.

More recent studies of maturity effects on fibrous components as analyzed by the methods of Van Soest (1963a) have been reported by Ingalls et al. (1965) and Ademosum et al. (1968). Although the fiber values reported as acid-detergent fiber are higher than those for cellulose and crude fiber, they follow the same trends, increasing with advancing maturity. The study of Ingalls et al. (1965) showed that fiber content ranged from 27 to 40%, with grasses generally containing less fiber than legumes, when considering forages of the same year and cutting. The work of Ademosum et al. (1968) involved a sorghum-sudangrass hybrid, a forage on which data relative to nutritive value was rather limited. It was found that acid-detergent fiber and cellulose increased sharply after the stage of 30 to 40% head emergence, while lignin content increased steadily up to this time and then leveled off.

These workers also found that lignification of the fiber (L/F) and cell wall (L/W) followed maturity more closely than did the simple concentrations of fiber and cell wall constituents. The cell wall constituents fraction includes hemicellulose, cellulose, and lignin (Van Soest and Wine, 1967). This component also increases with advancing maturity and generally represents 50 to 70% of the plant dry matter.

In summarizing the effects of advancing maturity on the fibrous components of forage plants, it can be stated that they all increase as maturity proceeds. However, cellulose sometimes decreases slightly during later stages of maturity. The rate of increase is gradual until heading at which time it increases rapidly and then tends to level off somewhat. The rate of fiber and lignin formation may also vary between species of forage as well as between varieties within a species, although these differences are usually smaller.

Protein content of grasses and legumes is also decreased with advancing maturity, this effect may have greater significance than the increase in fibrous components since protein is the most costly item which is supplemented in livestock rations. Numerous studies have shown that crude protein content of grasses decreases with maturity. A few of these studies include Phillips et al. (1954), Lloyd et al. (1961) and Colovos et al. (1961). All of these studies indicated that protein content of forages is highest during the vegetative stage of growth and gradually decreases throughout the season. Work by Fulkerson et al. (1967) showed that crude protein yield in grasses was highest

at the heads emerged stage. The failure of grasses to show any increase in protein after heading is presumably due to their determinate habit of growth. At that stage they are producing few new cells; however, new cellulose and other carbohydrates are being laid down as cells elongate. Their work also indicated that the rate of depression was more pronounced with the grasses than with alfalfa, especially during the early growth stages. The results of a study reported by Colovos et al. (1961) showed that early-cut bromegrass hay grown under 225 kg. nitrogen fertilization per hectare was equal to legume hay as a protein source. The results further showed that while late-cut bromegrass hay increased the yield of dry matter per hectare and consequently the digestible energy by about 10%, the digestible protein content decreased by more than 30%. Work by Blaser (1964) also showed that nitrogen fertilization increased the protein content of grasses and that digestibility remained higher at later stages of maturity. A study by Kivimae (1966) showed that the yield of digestible protein per hectare was at a maximum before heading and decreased rapidly in late stages of maturity. In addition, it was shown that crude protein decreased linearly at the rate of 0.27 percentage units per day. Additional studies by Heaney et al. (1966) with timothy, Brown et al. (1968) with orchardgrass and timothy and Ademosum et al. (1968) with a sorghum-sudangrass hybrid all showed that as plants mature the crude protein content is decreased. The study of Fulkerson et al. (1967) showed that cutting at the head emergence stage resulted in the harvest of only 70% of the possible

dry matter yield but 82% of the *in vitro* digestible dry matter and 94% of the crude protein. This clearly emphasizes the need of early harvest to obtain high yields of quality forage.

The changes which occur in the components of plants markedly affect the digestibility of the intact plant. In most all cases the increase in fibrous components and lignin and the decrease in protein content causes lowered digestibility. Sullivan (1955) in studying true cellulose and natural cellulose found that as season advanced cellulose content increased and its digestibility decreased. This observation was supported by the study of Kamstra *et al.* (1958) who found that stage of maturity at harvest had a marked effect on the IVCD of orchardgrass, alfalfa and timothy. It was also shown that the effect of maturity diminished greatly when the cellulose was isolated from the plant either as holocellulose or cellulose.

Further studies of maturity effects on cellulose digestibility have been conducted by Lloyd *et al.* (1961) and Tomlin *et al.* (1965). These studies also showed that cellulose digestibility is decreased by advancing maturity. The study of Tomlin *et al.* (1965) indicated that lignification was linearly related to cellulose digestion; however, this relationship did not exist for alfalfa.

Cellulose digestibility usually does not decrease rapidly until the heads emerge from the boot of grasses or about mid-bloom in legumes. After this period digestibility drops rapidly until seed development at which time it levels off.

Dry matter content of forages increases with advancing maturity while the digestibility of dry matter decreases. Digestibility of dry matter is probably the most often used criteria in assessing forage quality. It has the advantage of including all of the nutrients present in the forage rather than a single component such as cellulose or crude fiber. Organic matter and energy digestibility are also used and will be included in the review of maturity effects on digestibility.

Studies by Minson et al. (1960a, 1960b) showed that digestibility remained at a high and relatively constant level until the time when the heads began to emerge. The grasses used in these studies were S37 cocksfoot (orchardgrass), S23 ryegrass and S24 ryegrass. From the time of heading digestibility declined about one half unit per day. Although S37 cocksfoot and S24 ryegrass were of similar maturity, S37 had a consistently lower digestibility than S24. This pattern of digestibility was followed for both years of the study. These workers pointed out that it is only when stems and flowering heads are allowed to mature that they lead to a marked depression in digestibility. Due to the rapid fall in digestibility of forages after flowering, yield of digestible dry matter per hectare rises much more slowly than does yield of total dry matter. Murdock et al. (1961) using orchardgrass fed fresh to yearling heifers obtained results similar to the above workers. Digestibility decreased from 76% for vegetative samples to 55% for samples obtained when plants were in full bloom, a decrease of 27%.

Kivimae (1966) found that timothy produced optimum dry matter yields between early flowering and full flowering, while the highest yield of energy was harvested at early flowering. Organic matter digestibility decreased at the rate of one half unit per day, a value similar to that obtained by Minson et al. (1960b) for cocksfoot and ryegrass. An additional study utilizing timothy was made by Heaney et al. (1966). Four varieties were used to determine the energy availability at progressive stages of maturity. Energy digestibility decreased from about 70% at the prehead stage to 50% at the full bloom stage or slightly beyond. Digestible dry matter yields for all varieties were at a maximum at early heading. For two of the varieties studied increases in dry matter yield after heading failed to offset decreases in digestibility, resulting in lower digestible dry matter yields. For a third variety, the gains in dry matter yield after heading approximately balanced losses due to the decrease in digestibility, and digestible dry matter yield remained relatively constant at advanced growth stages. The authors pointed out that the rate of decline in digestibility of these grasses was not merely a function of time but was affected by year to year fluctuations in growing conditions. A dry period in 1962 increased the time required to reach full bloom, whereas in 1963 more regular rainfall resulted in more rapid growth and earlier maturity. These differences were reflected by a more gradual decline in digestibility in 1962. Brown et al. (1968) also found that growing conditions affect digestibility since orchardgrass

and timothy harvested on comparable dates were more digestible in 1963 than the following year.

A comprehensive study of bromegrasses by Wright et al. (1967) showed a sharp decline in digestibility values with advancing maturity in the first crop and small changes in the aftermath. Their study also indicated that dry matter production per hectare increased over all growth stages, whereas digestible dry matter per hectare did not increase after early bloom.

Considerable interest has been shown in recent years in varietal differences within species as to maturity, digestibility and nutritive value. Mowat et al. (1965b) using timothy, orchardgrass, bromegrass and alfalfa with two varieties of each representing a range in maturity or plant type found that in vitro dry matter digestibility remained essentially constant in early spring and then decreased rapidly. Orchardgrass had the lowest digestibility on a given date but the highest digestibility of any species when compared at the heading stage. Data obtained by Dent and Aldrich (1966) showed that cutting when 50% of the heads had emerged resulted in a tendency for early heading varieties to show a higher digestibility than later heading ones. This may be due to the fact that tissues of later varieties are more mature at a corresponding growth stage than those of early varieties.

Christie and Mowat (1968) in studying the variability of in vitro digestibility of bromegrass and orchardgrass clones also found later maturing plants to be less digestible than early maturing plants

when harvested at the same stage of maturity. They pointed out that it may be more difficult to develop a late-maturing variety as high in digestibility as an early-maturing one when both are to be harvested at the same stage of maturity. A study of climatological factors by Denium et al. (1968) may explain the above effects of later maturity. These workers calculated from their results that the approximately ten units lower digestibility of late-maturing plants compared to early-maturing plants is caused by both increased temperature and stem formation. They also stated that vegetative grass in mid-summer will have an approximately seven units lower digestibility than in early spring due to the higher temperatures.

In summarizing the effects of advancing plant maturity, it can be stated that the fibrous components and lignin increase rapidly as heading and flowering occur and then tend to level off. Protein content is highest in the early vegetative stages of growth and decreases more gradually throughout the season. Digestibility of the various components remains relatively stable during vegetative growth stages with a marked depression occurring at heading and flowering followed by a leveling off. It is also apparent that management systems recommended to produce high quality forage may involve the acceptance of reduced dry matter yields. It is evident that one must compromise between quality and quantity.

Effects of Advancing Maturity on Important Components and Digestibility of Plant Fractions

Attempts to predict forage nutritive value have also included separation of the plant into various fractions with the hope that chemical composition and/or digestibility of one fraction might be a better indicator of quality than the whole plant. Some of these studies have included the leaf sheath as a portion of the leaf while others have included it with the stem. The latter appears more appropriate although separate analysis of the sheath would be preferred. Data pertaining to the changes in components of plant parts with advancing maturity are rather limited when compared to the data available on *in vitro* digestibility of these fractions.

The percentage of the plant dry matter composed of leaf tissue has probably had more attention than any other fraction since leafiness has long been associated with high quality. Pigden and Heinrichs (1957) separated clonal lines of intermediate wheatgrass into leaf and stem (including sheath) fractions. Analysis of variance established significant differences in percent lignin between years. Plants contained the most lignin in the driest years. It was also shown that the clonal line with the finest stems had the lowest lignin content; however, the percentage leaf had a greater effect on lignin content than any other factor studied. Reid *et al.* (1959) found that the relationship between leaf content and dry matter digestibility for first growth forage was different from that for aftermaths. Aftermath forages ranging in leaf content from 55 to 92% were not much different

in dry matter digestibility, while leaf content and dry matter digestibility of first growth forage were highly correlated (0.95). In this study the leaf included the blade and sheath.

Comparisons of the lignin content of plant parts of bromegrass, intermediate and crested wheatgrass by Sosluski *et al.* (1960) indicated that at heading and flowering stems were higher in lignin than leaves and heads were higher than stems. The results indicated that, at early growth stages, prior to heading strain differences tend to be small and inconsistent, while from heading to flowering these differences may become more evident. It was concluded that these strain differences at later growth stages were due to differences in proportion of leaves, stems and heads and their relative lignin content. Intermediate wheatgrass strains were more rapidly lignified during the early growth period but showed little or no increase after heading.

Jarrige (1960) showed that stems contained a much greater proportion of membranes than the leaves, particularly in legumes. The leaves consisted primarily of parenchyma with thin membranes while stems were composed almost entirely of supporting and connective tissue. It was also found that the proportion of membranes in the stem rose rapidly with age, particularly with alfalfa and red clover. It remained practically constant in the leaves of legumes but increased in the stems and sheaths of grasses.

Pritchard *et al.* (1963) used a number of species to study the *in vitro* digestibility of whole grasses and their parts at progressive stages of maturity. The *in vitro* digestibility for leaves, heads and

stem segments (including sheath) decreased with advancing maturity. The digestibility of the heads was at least as high as that of the leaves in the early part of the season but decreased to a greater extent as maturity advanced. Stems from the first cuttings of timothy and bromegrass had higher in vitro digestibility values than the leaves, but as season progressed, the digestibility of the stem decreased faster than leaf digestibility so that they were lower by the last cutting. These qualitative changes, together with the increasing proportion of stem to whole plant as maturity progresses, indicate that alterations in the stems of grasses exert the major influence upon differences in digestibility of the whole plant. Work by Terry and Tilley (1964) confirms the above statement. In their work it was shown that digestibility fell only slightly before head emergence. These patterns could be explained by the changes in the proportions of leaf blade, leaf sheath and stem as the plants matured, together with the rates of decline in the digestibility of the fractions. Leaf blade, the major fraction prior to head emergence, declined most slowly (0.2% per day) and stem, which predominated after head emergence, declined most rapidly (0.8% per day). In this study percentage of leaf was not an indication of the relative digestibility of timothy. Work by Brown et al. (1968) with timothy and orchardgrass is in disagreement with the above statement. They found a very high correlation between leaf percentage and digestibility. The discrepancy between these studies may be explained by the fact that the study of Brown et al. (1968) included samples harvested only in early spring,

stem segments (including sheath) decreased with advancing maturity. The digestibility of the heads was at least as high as that of the leaves in the early part of the season but decreased to a greater extent as maturity advanced. Stems from the first cuttings of timothy and bromegrass had higher *in vitro* digestibility values than the leaves, but as season progressed, the digestibility of the stem decreased faster than leaf digestibility so that they were lower by the last cutting. These qualitative changes, together with the increasing proportion of stem to whole plant as maturity progresses, indicate that alterations in the stems of grasses exert the major influence upon differences in digestibility of the whole plant. Work by Terry and Tilley (1964) confirms the above statement. In their work it was shown that digestibility fell only slightly before head emergence. These patterns could be explained by the changes in the proportions of leaf blade, leaf sheath and stem as the plants matured, together with the rates of decline in the digestibility of the fractions. Leaf blade, the major fraction prior to head emergence, declined most slowly (0.2% per day) and stem, which predominated after head emergence, declined most rapidly (0.8% per day). In this study percentage of leaf was not an indication of the relative digestibility of timothy. Work by Brown *et al.* (1968) with timothy and orchardgrass is in disagreement with the above statement. They found a very high correlation between leaf percentage and digestibility. The discrepancy between these studies may be explained by the fact that the study of Brown *et al.* (1968) included samples harvested only in early spring,

while the study of Terry and Tilley encompassed a wider range in maturity.

Comprehensive studies of plant fractions of timothy, orchardgrass, bromegrass and alfalfa have been made by Mowat et al. (1965b, 1965c). Cuttings were made at weekly intervals for 12 weeks and plants were separated into leaf and stem fractions with about two-thirds of the leaf sheaths of grasses remaining with the stem. At early growth stages the digestibility of the stems of grasses was higher than that of leaves. However, the rate of decline of digestibility of the stems was greater, similar to the finding of Pritchard et al. (1963). The in vitro digestibility of the leaves and stems of all grasses was somewhat similar at head emergence. These workers concluded that providing these grasses are cut at an early stage little or no progress could be made in improving their digestibility by selection for leafiness per se. However, if grasses are not harvested until later, there appears to be ample scope for improvement. Mowat et al. (1965c) also showed that crude protein in both leaf and stem fractions decreased with advancing maturity. The decline was greatest during early growth stages and tended to gradually level off later. The leaves of grasses contained about twice as much protein as the stems, while nearly three times as much protein was present in the leaves of alfalfa as in the stems.

Another study by Mowat et al. (1965a) in which reproductive shoots were separated into leaves 100% living, leaves 100% dead, leaves partially living, heads and stems (including leaf sheaths) showed that

digestibility of all fractions decreased with advancing maturity. The decline in leaf digestibility was greater for those leaves dead or partially dead, a finding in agreement with Burton et al. (1964). Dead leaves were low in digestibility even at the early cutting date; nevertheless, in more mature plants dead leaves were higher in digestibility than heads or stems. These workers as well as Pritchard et al. (1963) indicate that selection for leafiness when forages are harvested early is of little benefit; however, the digestible energy content of a mature plant should be increased by such selection or by selection for a more digestible stem since the stem may comprise 40 to 50% of the total plant dry matter at later stages of maturity.

Several studies have been conducted to determine the effects of day length and temperature on the development of grasses. A study by Bowman and Law (1964) indicated that leaf percentage was highest in plants grown at 29° C. and the highest percent leaf was produced during a 14 hr. day. Ryle (1966) found that the most marked effect of day length was on leaf length. Significant increases in lamina length in long days were obtained in all experiments, irrespective of species. Examination of the lower epidermis of these leaves indicated that the longer leaves had more cells and longer cells than leaves produced in short days.

In the studies of Mowat et al. (1965c) digestibilities of Saratoga and Canada bromegrass were similar although the Saratoga variety had a much coarser, wider stem. A study conducted by Mowat et al. (1967) to determine the effect of stem diameter of alfalfa and

bromegrass on the *in vitro* dry matter digestibility showed that stem diameter varied greatly with the largest differences occurring between cuts. Stems from first cut alfalfa were 46% wider than those of second cut alfalfa and the 20 wide-stemmed samples of bromegrass were 42% wider than the 20 narrow-stemmed samples. Stem diameter had little effect on either *in vitro* digestibility or chemical composition with both alfalfa and several bromegrass entries.

Generally the changes of components of plant fractions with advancing maturity are similar to those of the intact plant. That is fibrous components and lignin increase while digestibility and protein content decrease. Leaves are generally lowest in fiber content and highest in digestibility and protein content. Stems, on the other hand, are highest in fiber and lignin content and lowest in crude protein and digestibility. The leaf sheath and seedhead are usually intermediate, although the leaf sheath resembles the stem more closely in composition and digestibility. As pointed out, when forages are harvested at early stages of maturity, leafiness is not critical since stems remain relatively highly digestible until the early heading stage. It may well be that the plant breeder could make more progress by selecting for stems of high digestibility since they compose the largest percentage of plant dry matter after heading.

EXPERIMENTAL PROCEDURES

Grass Species and Varieties Studied

The grasses studied included Manchar and Sac bromegrass, Greenar and Oahe intermediate wheatgrass and Siberian and Nordan crested wheatgrass. Since varieties within a species differed very little in morphological characteristics, the following discussion is limited to general species characteristics.

Smooth bromegrass is a cool-season, perennial, sod forming grass. It has maintained itself in established stands in eastern and central South Dakota for 50 to 60 years in certain recorded cases, although it tends to become "sod-bound" and low producing unless fertilized or renovated. Its drought resistance and ability to withstand extremes in climatic conditions are well established. Under dry summer conditions it becomes dormant but resumes growth when moisture and temperature become more favorable in the autumn. Smooth bromegrass is a high yielding forage grass adapted to all soil types but makes its best growth on fertile sandy loam or silt loam soils with plenty of moisture. It is highly palatable both when grazed or used for hay. Digestibility and forage production are high when hay is cut after heads have emerged but before flowering.

Intermediate wheatgrass is a cool-season grass species introduced from Russia and was first recognized as a valuable forage grass at the South Dakota Agricultural Experiment Station in 1938. It is a high yielding forage with stems somewhat coarser than those of most bromegrass varieties, but many leaves are produced so a highly

satisfactory forage is produced. The forage is palatable to livestock when grazed or harvested early. Seedlings are vigorous and easily established. Under conditions of 36 to 38 cm. precipitation, intermediate wheatgrass does not maintain stands as well as crested wheatgrass.

Crested wheatgrass is a long lived, drought resistant bunch grass which makes its growth and provides the best grazing early in the season. Under conditions of 23 to 38 cm. average annual precipitation it has maintained stands for more than 40 years, although yields decline with advancing age of the stand. It is useful as a supplementary pasture to native ranges, particularly to depleted ranges so as to extend the green forage season. Under normal conditions a pasture of crested wheatgrass extends the grazing season an additional two weeks both in early spring and late fall. Crested wheatgrass normally flowers in early June and matures in early July. It should be utilized in the spring and fall when it is very palatable to all livestock. After it begins to mature in late June, it becomes coarse and unpalatable.

Information about the general species characteristics was obtained from the data compiled by Ross et al. (1966).

Establishment and Management of Experimental Plots

Experimental plots were located one mile north of Brookings on Agronomy Department Research Fields and were seeded on August 23, 1965. A split plot design of two replicates of 0.1 hectare plots of each variety were seeded. Growth during the fall of 1965 was limited due

to a lack of moisture; however, it was adequate to insure winter survival of the seedlings.

All plots were fertilized with 225 kg. of ammonium nitrate per hectare on March 30, 1966, before any growth had begun. An additional 225 kg. was applied in the spring of 1967.

Management of the plots included mowing of boundaries between plots and around borders. Weed control was not necessary in any of the years included in the study. In a portion of one replicate of Sac bromegrass a poor stand resulted in considerable weed infestation; however, this area was not sampled.

After completion of sampling in 1966 all remaining aftermath was cut and removed from the plots so as to not interfere with sampling in 1967. Some regrowth did occur, but care was taken not to include this material in samples collected in 1967.

Precipitation during the 1966 growing period was normal, while precipitation during fall and winter months was 6.9 cm. below normal. Precipitation from January 1 to June 1, 1967, was 6.4 cm. below average for that period, although precipitation in June, 1967, was 10.0 cm. above average for that month. As a result of these precipitation patterns, growth during the spring of 1967 was slower than during 1966.

Harvesting and Preparation of Forage Material for In Vivo and In Vitro Studies

Forages used in the in vivo digestion trial were harvested at two stages of maturity. The first stage of maturity was harvested when 50% of the heads had emerged from the boot and the second stage

of maturity was harvested 14 days thereafter. These harvest dates were selected because they encompass the period in which digestibility declines most rapidly. The respective harvest dates were as follows: Manchar bromegrass; June 10, 24; Sac bromegrass, June 13, 28; Greenar and Oahe intermediate wheatgrass, June 22, July 6; Siberian crested wheatgrass, June 11, 25 and Nordan crested wheatgrass, June 7, 21. As seen from the harvest dates 15 days elapsed between the first and second harvest of Sac bromegrass. An extremely hard rain prevented harvesting the second stage on June 27.

Forages were cut with a sickle type mower about mid-morning and allowed to wilt in the field until mid-afternoon unless rain threatened. The grasses were raked and loaded onto wagons and transported to a drying shed. Drying was under natural but protected conditions. All of the forages were dried for at least two weeks while those harvested first dried for a longer period. After drying, the forages were chopped to a length of 1 to 2.5 cm. by feeding the material into a Gehl field chopper. The chopped material was blown into wool sacks to prevent loss of leaves and fine material. It was felt that this would provide a uniform and representative sample of the material harvested. There appeared to be very little separation of coarse and fine material when forage was removed from the bags.

Forage samples used in the chemical composition and <u>in vitro</u> digestibility studies were also sampled at mid-morning. Samples were clipped approximately 5 cm. above ground level and care was taken to exclude material from the previous year's growth. Plant material used

in the study of chemical composition and digestibility of plant fractions was sampled every nine days beginning June 1, 1966, and continued through August 12. After collection, this material was stored at -18° C. until separation could be made. Generally separations were completed within 4 or 5 days after collection.

Plants were separated into leaf blade, leaf sheath, stem and seedhead fractions. A portion of the sample was also left as intact plant. All of the material used in the *in vitro* digestibility and chemical composition studies was dried at 40° C. in large forced draft ovens for 48 hr. After drying the weight that each plant fraction contributed to the total plant dry matter was determined.

Samples were initially ground to pass a 1 mm. screen in a No. 3 Wiley mill. The ground sample was mixed and reground to pass a 0.417 mm. screen in an Intermediate Size Wiley mill. Grinding of samples in this manner provided for more representative sampling and prevented separation of coarse and fine particles. Samples were stored in tightly closed glass bottles free from excessive temperature and direct exposure to sunlight.

In Vivo Digestion Trial Procedures

Twenty-four wether lambs weighing approximately 40 kg. were randomly allotted to the six test forages. The lambs were placed in individual pens measuring 4 x 5 feet. During the 10-day preliminary period the lambs were fed *ad libitum* to determine maximum intake. This amount of feed was reduced by 10% during the collection period to encourage consumption of all forage offered. Level of feed offered

was held constant between varieties within species but not between species. In spite of the fact that feed allowance was reduced by 10% in relation to that offered during the preliminary period, feed refusal did occur. The refused feed (orts) was collected, dried, weighed and analyzed and the nutrients contained therein subtracted from that offered so that digestibility figures were expressed on an "as consumed" basis.

Samples of the forage fed were obtained by taking grab-samples at each feeding during the collection period. These samples were composited and subsampled at the end of the collection period. Grinding and storage of these samples was similar to that of samples used in the *in vitro* study.

Fecal collections were made using a harness and canvas bag. The fecal collection bags were attached three days prior to the start of the collection period to allow the lambs to become accustomed to the harness. Fecal collections were made twice daily, prior to the morning and evening feeding. The fecal samples were immediately dried in a forced draft oven at 60° C. for 48 hr. The dried feces were composited for the length of the collection period, mixed and sub-sampled for analysis. Approximately 100 gm. of feces were ground to pass a 1 mm. screen.

Throughout the trial water, trace mineralized salt and dicalcium phosphate were fed *ad libitum*. The lambs appeared comfortable at all times and the harness and canvas bags did not appear to interfere with

normal activity. The lambs were not stanchioned during the feeding period since this practice did not contribute to feed wastage.

Chemical Analysis Procedures

Cellulose analysis was conducted by the method of Crampton and Maynard (1938) with the following modifications. A pretreatment of the sample by boiling in 1.25% sodium hydroxide was used and the supernatant liquid was decanted after centrifugation. In addition, all processes were carried out in 100 ml. centrifuge tubes and the residue was recovered in Gooch crucibles. Cellulose was calculated as loss on ignition.

The method of Van Soest (1963) was used to determine acid-detergent fiber and acid-detergent lignin content of the forage samples. The only change made in this procedure was the use of Gooch crucibles with an asbestos pad for filtering.

A.O.A.C. (1960) methods of analysis were used for the determination of crude fiber, crude protein, ether extract, ash and nitrogen free extract of the samples of feed and feces obtained in the digestion trial.

In Vitro Rumen Fermentation Procedures

In vitro dry matter digestibility was carried out by using a procedure based on that of Tilley and Terry (1963). Since minor modifications may affect the results of in vitro procedures the method used is outlined in detail. One-half gram of forage was weighed into a 100 ml. polyproplyene test tube which served as the fermentation

vessel. The water bath which was used as the incubator was adjusted to 39° C. and on the day of the run 25 ml. of McDougalls solution was added to each tube about 1 hr. prior to the addition of the rumen inoculum to allow wetting of the sample. In this study rumen inoculum was collected 2 hr. after feeding by removal of ingesta from a rumen fistulated steer and squeezing this material through eight layers of cheesecloth. The rumen fluid was transported to the laboratory and restrained through eight layers of cheesecloth and one layer of silk. The strained rumen fluid was then mixed with prewarmed McDougalls solution in a ratio of 1.5:1.0 and 25 ml. were added to the fermentation vessels. It was important to work rapidly during these stages to avoid chilling of the rumen inoculum. Carbon dioxide was bubbled through the inoculum mixture during addition to the test tubes and the solution was kept well mixed with a magnetic stirrer to prevent settling of suspended material. Immediately after the addition of the rumen inoculum the test tubes were stoppered tightly with Bunsen gas release valves. After 2 hr. of incubation the test tubes were swirled gently to resuspend the substrate which had risen to the top of the fermentation media. A gentle swirling motion was critical to avoid splashing of the material onto the sides of the test tubes. The test tubes were shaken every 8 hr. throughout the fermentation period following the first shaking at 2 hr.

On completion of the 48 hr. fermentation the tubes were removed from the water bath and 1 ml. of 5% mercuric chloride was added to stop bacterial action. Approximately 1 hr. was allowed to elapse prior to

centrifugation. The sides of the test tubes were washed with distilled water and a rubber policeman to remove adhering residue. The test tubes were centrifuged at 1500 rpm for 10 minutes and the supernatant drained off.

Following completion of the bacterial fermentation 50 ml. pepsin solution (Tilley and Terry, 1963) was added as the initiation of the second stage pepsin digestion. Care was taken to resuspend the fermentation residue for 48 hr. at 39° C. Tubes were swirled every 8 hr. during the pepsin digestion. At the completion of the 48 hr. digestion tubes were washed as described previously and centrifuged at 1500 rpm for 10 minutes. The supernatant liquid was poured off and the test tubes plus residue were placed in a drying oven at 90° C. for 24 hr. In addition to the test forage samples, three or four tubes were carried through the entire procedure with the exception that they contained no forage sample. These "blank" tubes served to give the residue of incubated inoculum.

In vitro dry matter digestibility (IVDMD) was calculated by the following formula:

$$\text{IVDMD} = \frac{100 \times \left(\text{sample dry matter} - \text{residue of fermentation} - \text{residue of inoculum}\right)}{\text{sample dry matter}}$$

In vitro cellulose digestibility was carried out in the same manner with the exceptions that the pepsin digestion was eliminated and the residue of bacterial fermentation was analyzed for cellulose by the method of Crampton and Maynard (1938) with the modifications outlined in the experimental procedures section. In vitro cellulose

digestibility (IVCD) was calculated by the following formula:

$$\text{IVCD} = \frac{100 \times \text{cellulose in sample} - \text{cellulose in residue of fermentation} - \text{cellulose in residue of digested inoculum}}{\text{cellulose in sample}}$$

In order to include protein digestibility as a criterion for evaluating breeders strains, a preliminary study was made by *in vitro* methods. The procedure involved the use of the *in vitro* dry matter digestibility procedure described earlier. The residue of the digestion was then removed from the fermentation tubes and subjected to Kjeldahl analysis (A.O.A.C., 1960). The residue of duplicate samples was combined to provide a sample of adequate size for analysis. Digestibility of protein *in vitro* was calculated by the following formula:

$$\text{Protein digestibility} = \frac{100 \times \text{protein in sample} - \text{protein in residue}}{\text{protein in sample}}$$

No correction was made for protein remaining in the digested blank as in the *in vitro* cellulose and dry matter digestibility procedures.

All statistical data presented were analyzed according to methods outlined by Steel and Torrie (1960).

RESULTS AND DISCUSSION

In Vivo and *In Vitro* Comparison of Grass Varieties Within Species at Two Selected Stages of Maturity

This study was conducted to determine the correlation between *in vivo* and *in vitro* methods of determining digestibility with the realization that use of *in vitro* methods of forage evaluation are more feasible in plant breeding work. Grasses used in the *in vivo* study were harvested at the same stage of maturity. The first harvest was made when 50% of the heads had emerged from the boot and the second harvest was made 14 days thereafter. These stages were selected since the decline in digestibility of grasses is greatest during this period, providing the greatest test for method comparisons.

Differences in chemical composition were small and inconsistent between varieties within a species as shown in table 1. Manchar bromegrass contained more protein and less of the fibrous components at the first stage of maturity, but these differences became smaller or were reversed at the second stage. Greenar and Oahe intermediate wheatgrass were similar in composition at both stages of maturity. Siberian crested wheatgrass contained more protein than Nordan at both stages of maturity and more crude fiber and acid-detergent fiber at the second stage. Gross energy content differed very little between varieties of bromegrass and intermediate wheatgrass while Nordan crested wheatgrass contained significantly more than Siberian.

The relatively high ash content of Manchar and Sac bromegrass at the second stage of maturity could be explained by hard rains

TABLE 1. CHEMICAL COMPOSITION OF FORAGES AT TWO STAGES OF MATURITY

	EE[1] %	CF %	CP %	Ash %	NFE %	CMC %	ADF %	ADL %	GE Kcal./kg.
				Stage I					
Brome									
Manchar[2]	3.6	23.4	23.2	10.5	39.3	27.9	30.3	4.1	4150
Sac	2.3	26.4	19.2	9.2	42.9	29.8	31.6	4.1	4060
Intermediate									
Greenar	3.3	27.8	16.9	7.8	44.2	34.2	36.1	5.1	4100
Oahe	3.4	31.0	16.6	7.9	41.4	34.2	36.7	5.1	4080
Crested									
Siberian	3.2	27.2	22.1	8.6	38.8	31.5	31.6	4.7	4175
Nordan	3.3	27.5	20.4	7.7	41.1	30.6	31.6	4.5	4310
				Stage II					
Brome									
Manchar	2.1	30.5	16.9	12.3	38.1	31.7	39.9	6.0	4020
Sac	2.4	29.7	16.8	11.4	39.7	31.4	38.9	5.5	3910
Intermediate									
Greenar	3.9	35.2	11.6	6.4	42.9	37.2	42.8	6.6	4045
Oahe	2.8	36.9	10.9	6.7	42.7	38.4	44.6	7.4	3980
Crested									
Siberian	2.9	34.8	15.3	7.3	39.7	36.8	41.8	6.8	4070
Nordan	3.4	31.5	14.7	8.4	42.0	33.6	39.9	6.1	4250

[1] EE = ether extract, CF = crude fiber, CP = crude protein, NFE = nitrogen free extract, CMC = Crampton and Maynard cellulose, ADF = acid-detergent fiber, ADL = acid-detergent lignin, GE = gross energy.

[2] Manchar and Sac bromegrass, Greenar and Oahe intermediate, Siberian and Nordan crested wheatgrass.

previous to the day of harvest which caused considerable soil to adhere to the forage. This was evident during the harvesting process and also when the grasses were chopped.

Crude fiber, cellulose, acid-detergent fiber and acid-detergent lignin content of all varieties increased significantly ($P < .01$) with later maturity. Crude protein decreased significantly ($P < .01$) at the second stage of maturity. These changes in chemical composition are similar to findings of Ingalls *et al.* (1965) and Brown *et al.* (1968). The decline in crude protein content was greatest for the intermediate wheatgrass varieties, followed by crested wheatgrass and bromegrass.

When species means were calculated, significant differences in chemical composition were present (table 2). Intermediate wheatgrass was significantly ($P < .01$) lower in crude protein content than either bromegrass or crested wheatgrass but contained more crude fiber, cellulose and acid-detergent lignin than bromegrass but not crested wheatgrass. Acid-detergent fiber content was significantly higher ($P < .01$) for intermediate wheatgrass than either of the other species. Nitrogen free extract did not differ significantly between species although it was highest in intermediate wheatgrass. The species differences present were probably due to inherent differences since all were sampled at equal stages of maturity. These results are in agreement with the study of Christie and Mowat (1968). These workers found that later maturing varieties or species were less digestible when harvested at the same stage of maturity. The intermediate wheatgrass varieties were approximately two weeks later in maturity.

TABLE 2. CHEMICAL COMPOSITION AND DIGESTIBILITY OF BROMEGRASS, INTERMEDIATE AND CRESTED WHEATGRASS[1]

	Significance level	Brome %	Intermediate %	Crested %
Chemical Composition				
Crude fiber[2]	.05	27.5[a]	32.7[b]	30.3[ab]
Crude protein	.01	19.0[a]	14.0[b]	18.1[a]
Cellulose	.01	30.2[a]	36.0[b]	33.1[ab]
Acid-detergent fiber	.01	35.2[a]	40.1[b]	36.2[a]
Acid-detergent lignin	.05	4.9[a]	6.1[b]	5.5[ab]
Nitrogen free extract	NS	40.0	42.7	40.4
Digestibility				
Dry matter	NS	53.5	49.6	52.6
Crude fiber	NS	56.7	54.3	53.0
Crude protein	.01	68.1[a]	62.8[b]	69.0[a]
Nitrogen free extract	.05	53.3[a]	47.3[b]	49.4[ab]
Total digestible nutrients	NS	51.4	49.0	50.6
Cellulose	NS	57.4	53.7	56.7

[1] Values shown are averages of two varieties at two stages of maturity.

[2] Means in a subset bearing a different superscript differ significantly.

The species differences were also related to the anatomical composition of the plants when harvested. The intermediate wheatgrass varieties contained approximately 30% more stem tissue than bromegrass and 10% more than crested wheatgrass at the 50% headed stage. These differences became more pronounced at the second stage. Since the stem and sheath contain more fibrous components and lignin, it would appear that plants containing more stem tissue would be less digestible. Mowat *et al.* (1965c) have suggested that for these reasons plant selection work should be based on the digestibility of stems or whole plants.

Digestion coefficients of the individual varieties at the two stages of maturity are shown in table 3. Varietal differences within a species for all *in vivo* criteria were nonsignificant. Some of the differences appeared large enough to be significant; however, variability between lambs fed the same variety of forage was quite large in some cases. The exact cause of this variation was not known although these lambs had been used in a faunation-defaunation study previously. The defaunated lambs were associated with faunated lambs for three weeks in an attempt to eliminate any differences in possible digestive ability.

The digestion coefficients reflected chemical composition rather closely. Manchar bromegrass contained slightly less fiber than Sac at the first stage of maturity and as a result was slightly more digestible. At the second stage of maturity Manchar and Sac were nearly identical in composition but Manchar remained slightly more digestible. Although nearly identical in chemical composition at both stages of maturity, Greenar and Oahe intermediate wheatgrass differed in digestibility. Oahe was more digestible than Greenar at the first stage of maturity while the reverse was true at the second stage, but the differences were not significant at either stage. Nordan crested wheatgrass was slightly but not significantly more digestible than Siberian at both stages of maturity. This difference was greater at the second stage of maturity and could be related to chemical composition data as Siberian contained more fibrous components and lignin at the second stage of maturity.

TABLE 3. DIGESTION COEFFICIENTS OF FORAGES AT TWO STAGES OF MATURITY

	DM[1] %	CF %	CP %	NFE %	DE %	TDN %	CMC %	IVDMD %	IVCD %
				Stage I					
Brome									
Manchar	63.3	61.9	75.3	64.8	62.6	61.1	65.4	72.3	66.5
Sac	54.7	60.2	67.0	60.0	55.5	56.1	61.5	72.2	64.6
Intermediate									
Greenar	56.8	57.8	69.4	55.2	54.8	55.6	58.2	69.9	69.6
Oahe	60.3	68.9	72.6	57.4	58.5	60.5	64.5	67.9	64.3
Crested									
Siberian	60.0	57.2	75.2	57.1	57.5	57.7	63.7	72.5	74.1
Nordan	61.9	62.9	72.3	62.0	61.2	61.0	64.2	72.5	71.6
				Stage II					
Brome									
Manchar	48.4	54.3	64.2	43.7	47.8	44.1	54.2	58.3	52.9
Sac	46.9	49.9	64.7	44.5	44.6	43.5	46.9	59.7	52.6
Intermediate									
Greenar	44.3	49.9	57.8	39.9	40.9	43.4	49.2	59.5	54.9
Oahe	37.1	40.4	51.1	36.5	33.0	36.6	42.8	57.5	49.9
Crested									
Siberian	42.4	45.7	65.2	34.9	38.9	40.4	48.3	60.6	55.4
Nordan	46.2	46.3	62.8	43.7	46.9	43.2	50.7	61.9	58.4

[1] DM = dry matter, CF = crude fiber, CP = crude protein, NFE = nitrogen free extract, DE = digestible energy, TDN = total digestible nutrients, CMC = Crampton and Maynard cellulose, IVDMD = in vitro dry matter digestibility, IVCD = in vitro cellulose digestibility.

Digestibility of all components was significantly ($P<.01$) lower at the second stage of maturity. The percentage decrease for some of the criteria were dry matter, 24%; crude protein, 15%; digestible energy, 28% and total digestible nutrients, 28%. These decreases are of the same magnitude as those reported by Brown *et al.* (1968), Wright *et al.* (1967) and Ademosum *et al.* (1968). This highly significant decline in digestibility of the major nutrients of forages emphasizes the need for early harvesting to obtain forage of high quality and nutritive value. When varieties and maturity stages were averaged, intermediate wheatgrass was lowest in digestibility of all components shown in table 2.

In vitro dry matter (IVDMD) and *in vitro* cellulose (IVCD) digestibility values for Greenar and Oahe were significantly different, $P<.05$ and $P<.01$, respectively. Bromegrass and crested wheatgrass varieties did not differ significantly in IVDMD or IVCD. *In vitro* digestibility values were considerably higher than corresponding *in vivo* values; however, the correlation between *in vivo* and *in vitro* values was 0.89 and 0.88, respectively, for dry matter and cellulose (table 4). These correlation coefficients are similar to those obtained by Baumgardt (1962b) but slightly lower than those obtained by Tilley and Terry (1963) and Barnes (1966).

Simple correlation coefficients between *in vivo* digestibility and *in vitro* digestibility and chemical composition are shown in table 4. Cellulose content was significantly ($P<.05$) negatively correlated with dry matter, cellulose and protein digestibility *in vivo*. All

TABLE 4. SIMPLE CORRELATION COEFFICIENTS BETWEEN IN VIVO MEASURES OF DIGESTIBILITY AND SEVERAL LABORATORY ANALYSES

	In vivo DMD	In vivo CD	Digestible crude protein
In vitro DMD	0.89	--	--
In vitro CD	--	0.88	--
Cellulose content	-.62*	-.60*	-.64*
Acid-detergent fiber	-.85	-.89	-.85
Acid-detergent lignin	-.88	-.88	-.84
Crude protein content	0.85	0.83	0.91
Percent leaf	0.80	0.83	0.73

* $P < .05$, all other values $P < .01$.

other correlations shown were highly significant ($P < .01$). Acid-detergent lignin and fiber gave much higher correlations with in vivo digestibility than cellulose or crude fiber, although crude fiber is not shown in table 4. The correlation coefficient between crude fiber and in vivo dry matter digestibility was -.60. The correlation coefficients shown are of similar magnitude as those obtained by Baumgardt et al. (1958) and Oh et al. (1966). Correlations between chemical composition can be expected to be high when grasses are being compared. When legumes are included in the calculations, the correlations are usually reduced.

Acid-detergent fiber and lignin are recommended for use rather than earlier cellulose, fiber and lignin analyses methods. It also appeared that in vitro procedures could be used to estimate in vivo digestibility as evidenced by the significant correlations between the two methods.

Since protein is an important economic and nutritional component of forage, the digestibility of this component becomes important to the plant breeder. The selection for and development of forage varieties having a high digestible protein content should certainly be encouraged. At present, the determination of protein digestibility involves the use of a digestion trial which requires considerable amounts of forage, in addition to being an expensive, laborious and time consuming process.

In view of the laborious process of determining protein digestibility *in vivo*, a preliminary study was conducted to ascertain the problems associated with determining protein digestibility *in vitro*. It was expected that the separation of microbial protein from plant protein would be the first problem encountered in determining protein digestion *in vitro* since microbial protein would contribute to the residual protein remaining after fermentation in the artificial rumen. This would lower the digestibility value obtained.

With the above problem in mind, it was decided to use the simplest method possible for determining protein digestibility *in vitro*; namely, one which eliminated the separation of microbial and plant protein. The procedure used was essentially that of the *in vitro* dry matter digestibility procedure previously described. The residue of the *in vitro* dry matter digestion procedure was analyzed for protein by A.O.A.C. (1960) methods and digestibility of the protein was calculated by subtracting the weight of residual protein from the protein in the sample and dividing by the latter. This value was taken

times 100 to arrive at percent digestibility. It should be noted that no correction was made for microbial protein remaining in the residue of digestion. The values obtained when a correction was made for microbial protein remaining in samples of incubated inoculum without substrate were unusually high. The values for *in vitro* protein digestibility are compared with *in vivo* values of the digestion trial in table 5.

The correlation coefficient obtained between *in vivo* and *in vitro* protein digestibility was 0.95 ($P < .01$). Differences in *in vivo* protein digestibility between varieties were substantiated by the *in vitro* method with the exception of crested wheatgrass varieties at the first stage of maturity. It was realized that the high correlation obtained was for a limited number of samples which covered a narrow range of maturity. The probability of maintaining this high correlation over a large number of samples of more varied maturity and plant type is not known and warrants further study.

The correlation of 0.95 between *in vivo* and *in vitro* methods is only slightly higher than the correlation of 0.91 obtained between crude protein content and *in vivo* protein digestibility as reported in table 4. Anderson (1967) obtained an over-all correlation coefficient of 0.97 between crude protein content of the total ration and the digestible protein content. The study involved 613 individual digestion studies with yearling wether sheep. All hay rations were fed for 477 observations while concentrate:forage ratio of 20:80, 40:60 and 60:40 (or a higher percentage concentrate) gave 28, 43 and 44

TABLE 5. A COMPARISON OF CRUDE PROTEIN DIGESTIBILITY BY *IN VIVO* AND *IN VITRO* METHODS

Variety	Stage I		Stage II	
	In vivo	*In vitro*	*In vivo*	*In vitro*
	%	%	%	%
Bromegrass				
Manchar	75.3	76.1	64.2	63.0
Sac	67.2	70.3	64.7	66.6
Intermediate				
Greenar	69.4	67.7	57.8	58.5
Oahe	72.6	69.2	51.1	55.9
Crested				
Siberian	75.2	73.6	65.2	65.7
Nordan	72.9	76.1	62.8	64.9

observations, respectively. Correlation coefficients declined somewhat when higher levels of concentrate were fed. Nevertheless, this study indicated there may be a limited need for developing an *in vitro* technique for protein digestibility of forages.

Since a correction factor for protein remaining in the incubated inoculum without substrate increased digestibility values unrealistically, it is apparent that procedures must be modified to yield meaningful results. The incubated inoculum undoubtedly contained some plant protein as well as soluble protein. The separation of these proteins from microbial protein in the incubated inoculum should result in a smaller but more exact correction.

Weller *et al.* (1958) developed a method for separation of bacterial protein from protozoal and plant protein by using the compound diaminopimelic acid. This compound is unique in that it is present in the cell wall of bacteria but not in protozoa or plant

substances of the feed. A study by Weller *et al.* (1962) showed that the amount of diaminopimelic acid-nitrogen remained rather constant in the rumen of sheep fed wheat hay (9% protein) but varied considerably in those fed alfalfa hay (18% protein). This study also indicated that protozoa did not at any time form more than a small part of the microbial nitrogen in sheep fed wheat hay; however, protozoal nitrogen accounted for 25 to 40% of the microbial nitrogen when alfalfa hay was fed.

It would therefore appear that a determination of the amount of bacterial nitrogen present before and after an *in vitro* digestion would not serve as a consistent correction factor. Either protozoal nitrogen would have to be determined or the protozoa excluded from the microbial inoculum.

From the literature surveyed it appeared that diaminopimelic acid is the compound best suited for the separation of bacterial and plant nitrogen. Although the isolation and determination of this compound does not lend itself to routine analysis at the present time, additional study could possibly overcome this problem.

The results of this study, although superficial, indicate possibilities for developing methods to determine protein digestibility *in vitro*. In addition to providing important information about forage protein quality, such a method would be an invaluable aid in studying protein metabolism of microorganisms. The demand to include a value for digestible protein on feed labels of commercial protein supplements should also provide impetus for developing an *in vitro*

method for determining protein digestibility. Further study in the development of such methods is certainly warranted and recommended.

Although intake per unit of metabolic size was not determined in this study, differences in consumption were apparent. The bromegrass and crested wheatgrass varieties were consumed at equal levels while intermediate wheatgrass consumption was considerably lower than the other species. The refused portion of forage by lambs being fed intermediate wheatgrass consisted of stems high in fiber content. These stem fragments were uniformly chopped and did not appear longer than stem fragments of other grasses but they were larger in diameter and appeared quite coarse. This suggests that intermediate wheatgrass should be harvested on or before heading to provide forage which would be consumed readily.

The results of this study indicated that digestibility differences which existed between the varieties tested were small and non-significant when harvested at the same stage of maturity. Differences which were present at one stage of maturity may not have existed at a more advanced stage or they may have been reversed.

Differences were shown to be significant when closely controlled *in vitro* techniques were used, while between animal variability reduced the probability of showing significant differences when such small numbers were used.

The sharp decline in crude protein content, increase in fiber and lignin and highly significant decline in digestibility of these grasses during the 14 day interval clearly point out that these grasses

should be harvested on or before the date of 50% head emergence to obtain a forage of high nutritive value. Harvesting of grasses at this stage of maturity does not produce optimum dry matter yields as shown by Fulkerson et al. (1967). They showed that at the heading stage grasses had stored 70% of their dry matter but 82% of their in vitro digestible dry matter and 96% of their crude protein yield. It is apparent that a compromise must be made between quality and quantity of forage harvested.

Chemical Composition and In Vitro Digestibility of Grasses as Affected by Advancing Maturity Throughout the Growing Season

The conventional digestion trial requires relatively large amounts of forage and is not suited to season long comparisons, which may be valuable in plant selection programs. Thus, in vitro digestibility methods and laboratory analyses were used throughout the remainder of the study.

Crampton and Maynard cellulose content for the varieties used is shown in table 6 for both years of the study. The cellulose content of all varieties increased significantly ($P < .01$) with advancing maturity. Cellulose content in late May was about 22% and increased steadily through late June. In early June a slight decrease occurred for all varieties followed by a gradual increase in late July and early August. The slight decline in cellulose content in early July could not be explained by any of the factors generally associated with maturity. Possibly the extremely high temperatures during this period decreased the amount of cellulose synthesis. A study by Denium (1966)

TABLE 6. CELLULOSE CONTENT OF GRASSES AS AFFECTED BY ADVANCING MATURITY

Date	Bromegrass		Intermediate		Crested	
	Manchar %	Sac %	Greenar %	Oahe %	Siberian %	Nordan %
			1966			
5-23	19.3	18.8	21.4	19.2	22.7	22.9
5-29	20.2	21.6	21.8	22.5	24.8	24.3
6-4	22.8	22.1	24.7	23.5	26.5	26.6
6-10	23.8	22.5	23.7	23.4	28.1	27.3
6-16	25.9	25.8	25.5	27.4	31.6	30.6
6-22	27.3	28.8	30.9	30.3	33.6	32.6
6-28	29.7	30.1	34.4	33.6	37.2	33.8
7-4	28.1	28.9	33.8	35.3	37.2	33.5
7-10	27.5	29.2	36.3	33.5	36.4	31.9
7-16	27.7	27.2	35.2	35.1	35.1	34.9
7-22	31.3	30.3	33.6	33.8	32.9	32.9
7-28	33.2	30.7	34.3	35.8	33.8	32.1
8-3	30.9	29.3	35.2	35.9	34.7	31.7
8-9	32.7	30.1	35.9	36.7	36.3	34.2
8-15	33.7	32.5	35.7	38.5	36.5	33.7
$\bar{x}$	27.6	27.2	30.8	30.9	32.5[1]	30.9
			1967			
5-23	23.6	22.5	22.0	22.5	23.4	22.9
5-29	21.8	22.6	22.1	21.6	22.1	25.1
6-4	23.5	24.0	22.4	20.8	21.5	21.7
6-10	24.8	28.3	26.5	25.8	24.9	30.4
6-16	28.5	26.5	27.2	27.7	25.2	32.0
6-22	28.3	27.9	30.5	28.9	27.1	27.5
6-28	30.6	30.0	32.0	33.5	30.6	30.1
7-4	28.9	30.6	32.5	30.9	28.5	28.6
7-10	28.6	29.7	31.6	30.2	32.6	32.2
7-16	29.2	29.3	31.9	29.8	28.6	30.7
7-22	32.9	31.3	31.9	29.4	32.8	30.7
7-28	30.2	32.3	35.2	31.5	31.4	30.6
8-3	32.1	35.4	35.3	36.2	33.5	33.9
8-9	34.3	35.1	35.6	36.7	32.3	32.3
8-15	33.9	35.2	36.2	35.5	33.5	34.8
$\bar{x}$	28.7[1]	29.4	30.2[1]	29.4	28.5[1]	29.6

[1] Variety means within a species differ significantly ($P < .01$).

indicated that during drought crude fiber content decreased. This may have been the cause of the lower cellulose content observed in both years of this study. The effect appeared more pronounced in 1966 than in 1967.

As shown in table 6 differences in cellulose content between varieties within a species were small and variable throughout the season. Analysis of variance of the data showed that Siberian crested wheatgrass contained significantly ($P<.01$) more cellulose than Nordan in 1966 while in 1967 the reverse was true. Bromegrass and intermediate wheatgrass varieties did not differ significantly in cellulose content during 1966; however, in 1967 Sac bromegrass contained significantly more cellulose than Manchar and Greenar intermediate wheatgrass contained significantly more than Oahe.

The difference in cellulose content of the crested wheatgrass varieties could be attributed to maturity differences in 1967 but not in 1966. Nordan reached the heading stage of maturity four days earlier than Siberian. Manchar bromegrass was three days earlier than Sac while the intermediate wheatgrass varieties reached the heading stage at the same date. When species means were calculated, bromegrass contained significantly ($P<.01$) less cellulose than intermediate and crested wheatgrass with no difference between the latter two. The lower cellulose content of the bromegrass appeared to be an inherent characteristic since the maturity was only slightly earlier than the crested and intermediate species. When harvested at a given date without regard to maturity, the intermediate varieties should be

lowest in cellulose content. However, even at early sampling dates intermediate varieties contained as much cellulose as the crested varieties which were about two weeks more mature. This may be related to the work reported by Denium (1968) in which it was found that later maturing forage was less digestible due to increased stem formation caused by higher temperatures. It was also shown that the increase in stem tissue caused an increase in the fibrous components.

The effect of year of harvest on cellulose content, although small and unimportant, was significant ($P < .01$). The means when all varieties and dates were averaged were 29.9% in 1966 and 29.3% for 1967.

From the data presented in table 6 it is evident that cellulose content of all varieties was higher in late May of 1967 than in 1966. This is apparently due to maturity differences since all grasses reached the heading stage of maturity about one week earlier in 1967 than in 1966. Thus, differences due to year of harvest may be due to the more advanced maturity of plants as well as climatological factors. It is also of interest that bromegrass varieties contained less cellulose in 1966 than in 1967 while intermediate and crested wheatgrass varieties contained more cellulose in 1967. No explanation could be given to account for this.

As will be pointed out in the plant fraction study, the lower cellulose content of bromegrass varieties can be related to the lower percentage of stem and sheath tissue compared to the other species. On a seasonal average intermediate and crested wheatgrasses contained 13 and 24% more stem tissue, respectively, than bromegrass.

Acid-detergent fiber (ADF) followed a pattern similar to that of cellulose content with advancing maturity. All grass varieties increased significantly in ADF with later sampling date (table 7). ADF and cellulose values were similar in early spring harvests but differed considerably later in the season due to the inclusion of lignin in the ADF fraction. Some workers have used the value obtained by subtracting lignin from ADF as the cellulose content of forages. ADF content of the grasses increased steadily until head emergence and then increased markedly through flowering and early seed development. The trend of a slight decrease in cellulose content in early July was also apparent for ADF content. This may be related to seed development which would be higher in starch and therefore decrease cellulose and fiber content of plants. The increase in ADF following the decline may have been due to subsequent shattering of the developed seed. Also, the contributing effects mentioned for cellulose may apply to ADF as well.

Varietal differences within species were small during both years of the study. Manchar bromegrass contained significantly more ADF than Sac in 1966, although the difference was not significant in 1967. Greenar intermediate wheatgrass contained significantly ($P < .01$) more ADF than Oahe in both years of the study. As shown in table 7, Siberian crested wheatgrass contained significantly more ADF than Nordan in 1966, but this difference was reversed in 1967, similar to the cellulose data.

TABLE 7. ACID-DETERGENT FIBER CONTENT OF GRASSES AS AFFECTED BY ADVANCING MATURITY

	Bromegrass		Intermediate		Crested	
Date	Manchar %	Sac %	Greenar %	Oahe %	Siberian %	Nordan %
			1966			
5-23	19.4	19.4	21.4	19.6	21.9	21.9
5-29	20.8	22.8	21.8	23.0	23.8	25.7
6-4	25.1	24.2	26.9	27.6	27.2	27.2
6-10	26.2	25.0	25.5	26.2	28.7	28.1
6-16	28.9	27.6	29.8	30.9	32.6	31.2
6-22	30.8	32.8	35.2	34.3	36.6	36.3
6-28	35.2	35.1	39.0	38.9	40.5	38.4
7-4	36.4	35.9	39.6	40.1	41.2	37.6
7-10	34.9	37.2	40.6	39.8	40.5	37.4
7-16	35.9	36.6	42.3	42.3	40.5	37.3
7-22	37.8	36.0	40.3	39.9	39.1	39.3
7-28	39.3	37.5	40.8	42.2	39.9	39.1
8-3	38.8	35.9	41.1	42.8	42.5	39.0
8-9	39.3	36.7	41.6	42.8	43.7	41.9
8-15	40.9	38.8	42.6	44.9	43.7	41.9
$\bar{x}$	32.6[1]	32.1	35.2[1]	35.7	36.2[1]	34.8
			1967			
5-23	24.3	23.3	22.2	25.6	24.5	24.5
5-29	23.5	25.1	24.5	23.9	23.6	25.5
6-4	25.6	27.1	26.3	24.3	24.5	30.4
6-10	28.8	31.3	29.9	26.8	27.9	32.1
6-16	34.6	30.6	31.7	30.3	29.9	37.8
6-22	35.6	33.5	32.9	33.6	31.9	35.3
6-28	34.9	34.8	36.5	35.7	35.5	35.6
7-4	35.6	35.2	37.5	36.7	36.2	36.3
7-10	37.9	38.2	39.3	37.4	37.9	38.2
7-16	38.3	36.4	38.4	36.6	37.9	37.5
7-22	40.2	37.7	37.1	37.1	35.5	35.6
7-28	40.4	40.9	39.1	37.8	36.1	36.7
8-3	38.7	40.9	40.1	39.2	36.5	37.2
8-9	41.9	41.3	40.9	40.5	37.9	38.1
8-15	42.6	42.3	41.8	41.2	37.4	39.2
$\bar{x}$	34.9	34.6	34.6[1]	33.8	32.9[1]	34.7

[1] Variety means within a species differ significantly ($P < .01$).

Species means computed for both years of the study showed that bromegrass was significantly ($P < .01$) lower in ADF content than intermediate or crested wheatgrass. The latter two species were nearly identical in ADF content when the two years were combined. The lower ADF content of bromegrass was probably due to anatomical composition since it contained considerably less stem tissue than the other species during the growing season. It should also be pointed out that, although the intermediate wheatgrasses were two weeks later to mature, ADF content was similar to or higher than earlier maturing species. This was also true for cellulose content, indicating that later varieties and species of grasses mature more rapidly than earlier ones.

As shown in table 7, the ADF content for all varieties was higher in plants sampled in May of 1967 than in 1966. This trend remained in June for bromegrass and intermediate varieties but not for crested wheatgrass varieties. These differences early in the growing season were probably due to an earlier maturity in 1967 since the average date of heading was nearly one week earlier.

Acid-detergent fiber has been shown to be an excellent indicator of forage quality by Van Soest (1963b). ADF represents a reasonably pure lignocellulose and its composition is of nutritional significance. It appears to be a better indicator of nutritional value than cellulose or crude fiber due to the fact that the values continue to increase throughout the growing season compared to the leveling off observed with cellulose and crude fiber values. Since it includes lignin, it encompasses more of the fibrous, less digestible components of grasses.

Analysis of variance of acid-detergent lignin (ADL) content data indicated a significant ($P < .01$) increase with later sampling date (table 8). ADL content, like cellulose and ADF, increased most rapidly during the heading and flowering stages of maturity. The slight decline noted in cellulose and ADF content in early July was also present in the ADL values, although this was more pronounced in 1966.

Greenar intermediate and Siberian crested wheatgrass contained significantly ($P < .01$) less ADL than Oahe intermediate and Nordan crested wheatgrass during 1966. These were the only significant differences present between varieties within species during both years of the study. In both of these species varietal differences were probably not due to maturity differences since the intermediate varieties were similar in stage of maturity and Nordan was earlier to mature than Siberian crested wheatgrass.

The values shown for ADL may appear to be low; however, the method of Van Soest (1963b) yields values within this range. It appears that the material obtained in previous lignin analysis methods of Crampton and Maynard (1938) and Ellis et al. (1946) contained considerable amounts of plant proteins and other impurities. Values obtained by the method of Ellis et al. (1946) ranged from 10 to 20% while those obtained by Van Soest (1963b) ranged from 2 to 10%.

When varieties were averaged over both years of the study, it was found that crested wheatgrass contained significantly ($P < .01$) more ADL than intermediate wheatgrass and bromegrass. Mean ADL contents of the species were 6.2, 5.4 and 5.6%, respectively. The higher ADL

TABLE 8. ACID-DETERGENT LIGNIN CONTENT OF GRASSES AS AFFECTED BY ADVANCING MATURITY

	Bromegrass		Intermediate		Crested	
Date	Manchar %	Sac %	Greenar %	Oahe %	Siberian %	Nordan %
			1966			
5-23	1.7	1.6	2.0	1.5	2.5	2.3
5-29	2.0	2.5	2.2	2.6	3.2	2.9
6-4	3.1	2.9	3.1	3.8	3.7	4.2
6-10	2.7	2.9	2.6	3.1	3.4	3.6
6-16	3.8	3.1	3.8	3.9	4.9	4.7
6-22	4.7	4.7	4.8	5.2	4.8	4.8
6-28	5.0	5.2	5.6	5.8	7.6	6.4
7-4	5.7	5.9	6.4	6.7	7.3	6.8
7-10	4.9	5.4	5.3	5.6	5.7	5.9
7-16	5.4	5.4	6.0	6.2	7.6	6.1
7-22	6.0	6.4	6.3	6.2	7.3	7.3
7-28	6.2	5.9	6.8	6.6	7.1	7.4
8-3	6.5	5.5	5.8	6.9	6.8	6.6
8-9	7.1	6.7	6.4	6.9	8.2	7.3
8-15	7.1	7.1	7.4	8.2	8.7	7.9
$\bar{x}$	4.8	4.8	4.9[1]	5.3	4.9[1]	5.6
			1967			
5-23	3.5	3.4	3.2	3.4	4.0	3.4
5-29	3.4	3.5	3.3	4.2	5.6	3.9
6-4	4.4	4.4	4.5	4.5	4.7	4.3
6-10	4.3	4.6	5.0	4.4	4.1	4.2
6-16	6.3	6.0	4.7	4.9	5.7	5.7
6-22	5.9	6.0	5.3	4.9	4.9	6.6
6-28	6.1	6.0	5.4	4.6	5.9	6.1
7-4	7.1	6.1	6.2	4.8	6.6	6.2
7-10	7.0	7.3	6.6	7.1	7.2	6.9
7-16	7.4	6.4	6.4	6.8	8.6	8.4
7-22	6.5	7.1	6.6	5.8	8.3	7.6
7-28	7.4	7.4	6.2	6.4	7.3	7.2
8-3	7.0	7.3	6.2	8.2	8.1	8.2
8-9	8.2	10.1	8.4	8.3	9.0	10.1
8-15	9.3	9.9	8.1	7.6	8.7	8.6
$\bar{x}$	6.3	6.4	5.7	5.7	6.6	6.5

[1] Variety means within a species differ significantly ($P < .01$).

content of crested wheatgrass may be due to its earlier maturity. This was substantiated by the results of the *in vivo* study in which plants were harvested at the same stage of maturity. Under these conditions intermediate wheatgrass contained the most ADL followed by crested wheatgrass and bromegrass.

Differences in ADL between years were highly significant ($P<.01$). Mean ADL content in 1966 was 5.2% compared to 6.2% in 1967. The higher ADL content in the second year may have been due to the earlier maturity of all varieties, although it is doubtful that this was the only contributing factor.

Like ADF, ADL has also been shown to be highly correlated with forage digestibility. Oh *et al.* (1966) obtained correlations ranging from -.66 to -.95 within forage species between ADL content and *in vivo* dry matter digestibility. The *in vivo* study reported earlier showed a significant correlation of -.88 between ADL and *in vivo* dry matter digestibility. When forages of different species were compared, the correlation between ADL and digestibility was reduced. This was apparently due to the differential effect of ADL on certain components between species.

In vitro dry matter digestibility (IVDMD) of the grass varieties is shown in table 9. Before any discussion of these data is presented, it should be noted that all of the values shown were obtained in the same *in vitro* fermentation. The same rumen inoculum was used for all samples so that the differences which existed were not due to changes in microbial activity often associated with *in vitro* studies. This

TABLE 9. *IN VITRO* DRY MATTER DIGESTIBILITY OF GRASSES AS AFFECTED BY ADVANCING MATURITY

Date	Bromegrass		Intermediate		Crested	
	Manchar %	Sac %	Greenar %	Oahe %	Siberian %	Nordan %
			1966			
5-23	80.1	79.2	79.7	79.2	79.4	80.4
5-29	81.3	81.2	79.2	79.2	77.6	77.0
6-4	79.0	76.9	77.2	76.8	74.9	74.?
6-10	76.0	76.0	78.0	76.5	76.1	75.7
6-16	74.2	73.9	73.1	72.3	71.1	71.4
6-22	65.3	65.9	71.0	69.3	68.2	62.0
6-28	64.2	66.6	64.4	66.5	65.9	64.2
7-4	59.4	60.4	60.5	60.9	57.2	57.5
7-10	61.6	60.8	59.9	57.7	55.5	58.3
7-16	61.1	60.6	58.1	57.9	53.8	55.9
7-22	59.9	62.2	64.0	61.7	54.3	55.3
7-28	58.8	60.2	61.3	56.9	53.5	53.8
8-3	56.6	57.2	58.9	58.1	52.2	55.8
8-9	58.1	58.4	58.7	56.7	51.2	54.0
8-15	54.3	57.4	55.4	56.8	51.4	53.3
$\bar{x}$	65.9	66.5	66.7[1]	65.8	62.8	63.3
			1967			
5-23	73.2	74.3	77.4	74.9	76.4	74.2
5-29	74.3	73.2	74.9	74.8	73.4	74.6
6-4	70.8	69.9	71.3	75.3	73.3	71.8
6-10	70.4	66.9	70.6	70.7	70.7	68.9
6-16	63.1	66.8	68.5	71.3	68.6	65.3
6-22	61.0	63.0	66.5	67.5	66.8	66.2
6-28	60.1	61.8	69.9	67.6	64.4	63.2
7-4	60.4	63.9	71.6	66.8	62.6	61.8
7-10	57.0	58.3	62.6	56.9	62.3	58.6
7-16	52.0	59.4	60.3	60.5	55.2	57.8
7-22	51.3	54.5	60.1	60.1	56.2	57.7
7-28	49.2	50.0	58.2	58.1	53.8	56.1
8-3	49.9	49.0	57.2	58.6	55.1	52.9
8-9	46.4	50.4	53.8	54.5	50.6	51.9
8-15	47.3	49.4	55.5	51.9	49.9	51.9
$\bar{x}$	59.1[1]	60.7	65.2	64.6	62.6	62.2

[1] Variety means within a species differ significantly ($P<.01$).

involved the use of 360 fermentation vessels and two large water baths. The samples were randomly placed in one of the two water baths. The temperature of the two water baths did not differ by more than 0.5° C. during the 48 hr. fermentation.

IVDMD declined significantly ($P<.01$) with later sampling date for all varieties (table 9). As seen in the table, IVDMD declined slowly until the heading stage of maturity and then declined sharply during flowering and early seed development. After this period IVDMD declined more slowly and tended to level off during late July and early August. The decrease of fiber, cellulose and lignin in early July which was discussed earlier was not reflected in the digestibility data. It would be expected that the decrease in these components would cause a slight increase in IVDMD; however, this did not occur although there appeared to be a definite plateau in the rate of decline of IVDMD during this period. The decline in IVDMD with advancing maturity of grasses has been shown by many workers including Mowat et al. (1965a) and Fulkerson et al. (1967).

Differences in IVDMD between varieties of the same species were small throughout the growing season. Greenar intermediate wheatgrass was significantly ($P<.01$) more digestible than Oahe in 1966 but not in 1967. Sac bromegrass was significantly more digestible than Manchar in 1967 but not in 1966 although the trend was similar. The intermediate wheatgrass varieties were remarkably similar in IVDMD throughout the growing season, a reflection of their chemical composition. Nordan crested wheatgrass was slightly but not significantly more

digestible than Siberian in 1966. If these varieties had been sampled at the same stages of maturity, Nordan probably would have been more digestible than Siberian as was found in the *in vivo* digestion trial.

Species means computed by averaging varieties and harvest dates showed intermediate wheatgrass to be significantly ($P < .01$) more digestible than the other two species. There was no difference in the digestibility of bromegrass and crested wheatgrass. The higher digestibility of the intermediate species could be related to its later maturity when sampled at the same date. When these species were evaluated at an equal stage of maturity in the *in vivo* digestion trial, intermediate was lowest in digestibility. This points out the necessity of evaluating plants at equal stages of maturity to obtain data which are comparable. Species means for IVDMD were 63.1% for bromegrass, 65.6% for intermediate wheatgrass and 62.7% for crested wheatgrass.

IVDMD values were significantly ($P < .01$) lower in 1967 than in 1966. The mean IVDMD was 65.2% and 62.4%, respectively. Again, this difference could be attributed to maturity differences which existed between years as well as climatological factors. For these reasons it appears that the expression of digestibility declines as percentage units per day or fractions thereof may be misleading since specific stages of maturity are not reached at the same time each year. Kivimae (1966) and Minson *et al.* (1960b) have utilized this method of expressing the decline in digestibility. The use of this method

presents the decline in digestibility as a uniform trend throughout the season and in reality this does not occur. The rate of decline is gradual until emergence of the seedhead after which digestibility declines rapidly for several weeks and then tends to be at a more gradual rate after this period.

IVDMD has consistently given excellent correlations with *in vivo* digestibility. Studies by Oh *et al.* (1966) and Barnes (1966) showed that IVDMD provided the best estimate of *in vivo* dry matter digestibility when several chemical analyses and *in vitro* procedures were compared. Correlations obtained by these workers were 0.88 and 0.97, respectively. The correlation of 0.88 is similar to that obtained in the *in vivo*-*in vitro* study reported in the preceding section. The IVDMD procedure of Tilley and Terry (1963) is widely used for forage evaluation purposes. Although numerous minor modifications of the procedure have been made to facilitate its use in different laboratories, the results obtained are quite similar as evidenced by the high correlations obtained.

Generally, *in vitro* cellulose digestibility (IVCD) exhibited the same trends as IVDMD. Again, all values being compared in table 10 were obtained in the same *in vitro* fermentation. IVCD also decreased significantly ($P < .01$) with later sampling date. The decline in IVCD from late May to August was greater than the decline in IVDMD. This probably is due to the encrusting effect of lignin on cellulose directly. Studies by Bolker (1963) and Sullivan (1966) indicated that there may be a direct linkage between lignin and hemicellulose. If

TABLE 10. IN VITRO CELLULOSE DIGESTIBILITY OF GRASSES AS AFFECTED BY ADVANCING MATURITY

	Bromegrass		Intermediate		Crested	
Date	Manchar %	Sac %	Greenar %	Oahe %	Siberian %	Nordan %
			1966			
5-23	85.4	85.9	86.9	87.9	84.3	85.2
5-29	82.9	79.9	86.5	84.0	81.3	80.5
6-4	76.3	79.3	83.9	79.7	78.9	78.3
6-10	77.8	79.6	83.9	81.9	79.5	79.3
6-16	70.4	73.6	77.1	75.7	74.1	71.7
6-22	63.7	62.9	69.4	71.7	66.9	63.8
6-28	57.9	62.9	67.3	67.9	58.2	59.4
7-4	48.4	53.0	56.7	57.6	49.5	53.8
7-10	47.7	52.6	60.7	61.3	48.5	48.2
7-16	50.6	52.0	58.2	58.0	46.0	51.3
7-22	53.1	58.0	62.5	57.5	44.8	48.9
7-28	49.9	54.9	58.9	54.7	40.9	42.8
8-3	46.4	52.3	55.9	55.1	39.4	40.7
8-9	47.9	46.4	57.6	49.9	38.2	40.4
8-15	53.9	51.8	61.2	54.1	40.9	40.6
$\bar{x}$	60.8[1]	63.0	68.4[1]	66.5	58.1	58.9
			1967			
5-23	73.9	75.1	77.5	71.4	70.8	75.4
5-29	74.2	75.4	77.6	80.9	77.3	76.6
6-4	75.1	68.8	72.7	75.0	72.8	63.9
6-10	67.9	65.5	74.4	75.9	68.9	58.0
6-16	57.4	63.9	71.1	70.3	61.9	60.8
6-22	49.7	57.1	70.0	68.1	58.1	50.4
6-28	50.1	52.8	67.4	69.9	55.0	44.5
7-4	45.3	55.5	62.1	62.1	44.6	46.9
7-10	41.4	44.1	58.4	60.6	53.0	43.8
7-16	41.1	49.2	55.1	57.3	39.0	42.1
7-22	42.0	48.7	54.0	48.7	44.7	44.5
7-28	35.1	38.2	58.6	53.7	32.0	40.7
8-3	36.1	39.3	52.3	49.7	39.3	47.5
8-9	34.9	40.3	52.7	52.5	40.6	39.5
8-15	45.9	48.7	56.8	51.9	47.7	48.6
$\bar{x}$	51.4[1]	54.8	64.1	63.2	53.7	52.2

[1] Variety means within a species differ significantly ($P < .01$).

this is the case, it would be expected that as lignin increased cellulose digestibility would be decreased more than dry matter digestibility. IVCD followed a pattern similar to IVDMD in that the largest decrease occurred during the heading to early seed development stage of maturity. In contrast to IVDMD, IVCD continued to decline gradually following seed development and reached a lower level than IVDMD.

Greenar intermediate wheatgrass was significantly ($P<.01$) more digestible than Oahe in 1966 and Sac bromegrass was more digestible ($P<.01$) than Manchar during both years while the crested wheatgrass varieties did not differ significantly. Since Greenar and Oahe intermediate wheatgrass matured at the same rate, the difference in IVCD was due to inherent characteristics. When these two varieties were compared in the in vivo digestion trial reported previously, Greenar was also more digestible than Oahe. Manchar bromegrass was more digestible than Sac when compared at similar stages of maturity, but as mentioned above, when sampled at a given date Sac was more digestible.

Species differences for IVCD were considerably larger than for IVDMD. Intermediate wheatgrass was significantly ($P<.01$) more digestible than bromegrass and crested wheatgrass. Species means were bromegrass, 57.5%; intermediate wheatgrass, 65.5%; and crested wheatgrass, 55.8%. The means shown are directly related to maturity differences since intermediate wheatgrass was the latest maturing while crested wheatgrass was slightly earlier than bromegrass. As shown in table 10, digestibility of intermediate wheatgrass varieties did not

reach as low a level as the other species; however, this was not as apparent for IVDMD.

IVCD values were significantly lower ($P<.01$) in 1967 than 1966. Mean IVCD values were 62.7 and 56.6% for the two years, respectively. This difference could be accounted for by earlier maturity and increased lignin content of plants sampled during the 1967 growing season.

Interactions which were significant in the chemical composition and digestibility studies included the following: variety by date within species, species by year, variety by year within species, date by year and species by date by variety. The significant variety by date within species interaction indicates that one variety was not superior to another for all sampling dates studied. The interactions containing year effects were significant because of the variation in chemical composition and *in vitro* digestibility which existed from year to year for a given sampling date. These effects could all be related to the differences in maturity which existed between the two years of the study. The significant interactions substantiate the commonly held concept that varieties and species of forage do not exhibit similar chemical composition and digestibility values from year to year at a given sampling date. Date by year, species by year, species and year means are presented in appendix tables 1 and 2.

Year of harvest by variety interaction means are presented in table 11. As was pointed out previously, digestibility and chemical composition differences between varieties within species were small.

TABLE 11. EFFECT OF YEAR OF HARVEST ON CHEMICAL COMPOSITION AND *IN VITRO* DIGESTIBILITY OF SELECTED GRASS VARIETIES[1]

Year	Bromegrass		Intermediate		Crested	
	Manchar %	Sac %	Greenar %	Oahe %	Siberian %	Nordan %
			Crampton and Maynard Cellulose			
1966	27.6	27.2	30.8	30.9	32.5	30.9
1967	28.7	29.4	30.2	29.4	28.5	29.6
Mean	28.2	28.3	30.5	30.2	30.5	30.3
			Acid-Detergent Fiber			
1966	32.6	32.1	35.2	35.7	36.2	34.8
1967	34.9	34.6	34.6	33.8	32.9	34.7
Mean	33.8	33.4	34.9	34.8	34.6	34.8
			Acid-Detergent Lignin			
1966	4.8	4.8	4.9	5.3	5.9	5.6
1967	6.3	6.4	5.7	5.7	6.6	6.5
Mean	5.6	5.6	5.3	5.5	6.3	6.1
			In Vitro Dry Matter Digestibility			
1966	65.9	66.5	66.7	65.8	62.8	63.3
1967	59.1	60.7	65.2	64.6	62.6	62.2
Mean	62.5	63.6	65.9	65.2	62.7	62.8
			In Vitro Cellulose Digestibility			
1966	60.8	63.0	68.4	66.5	58.1	58.9
1967	51.4	54.8	64.1	63.2	53.7	52.2
Mean	56.1	58.9	66.3	64.9	55.9	55.6

[1] Mean of 15 harvest dates per year.

Differences which were present could usually be related to differences in maturity. It is apparent from this season-long study and the *in vivo* study that differences between varieties within species used in this investigation were minor and of little practical importance. The chemical analyses used and the *in vitro* methods appeared highly related since differences in chemical composition were reflected in IVDMD and IVCD.

This investigation also indicated that a season-long comparison may be more valid than comparisons based on only a few harvests. As seen in the tables, differences which are present at one stage of maturity may not be present at a more advanced stage. A season-long study may also be warranted to determine the ultimate level of digestibility which is reached late in the growing season. Studies of this nature should prove helpful to the plant breeder in assessing forage quality and in the selection of strains which remain high in digestibility throughout the season.

Chemical Composition and In Vitro Digestibility of Plant Fractions With Advancing Stages of Maturity

During recent years considerable research has been conducted in the area of laboratory evaluation of forages to determine their nutritive value. The majority of this work has utilized the whole plant, with little consideration given to the various plant fractions and how they may affect chemical composition and digestibility of the whole plant. This portion of the study was conducted to determine the chemical composition and IVDMD of certain plant fractions and their effects on the intact plant. The amount of dry matter contributed to the total plant dry matter by each fraction was also considered.

Samples of the six forage varieties were collected every ninth day beginning June 1, 1966, and continuing through August 12. Plants were separated into leaf blade, leaf sheath, stem and seedhead fractions. A portion of the sample was left as intact plant to allow comparison of plant fractions with the intact plant. Analysis of

variance of the data was conducted on a within species basis since all of the IVDMD samples could not be accommodated in one fermentation run. As the tables are examined, it should be kept in mind that samples were collected at a given date and not at equal stages of maturity. In order to avoid unnecessary repetition in discussing each species, a discussion of previous work in this area follows the results obtained in this study.

Bromegrass Varieties. Dry matter contribution of the various plant fractions to the total plant dry matter of Manchar and Sac bromegrass is shown in table 12. Leaf content of these two varieties was quite similar throughout the growing season even though Manchar was three days earlier in maturity. Leaf content declined at the rate of nearly 2 percentage units per day from June 1 to 19 after which time the decline was very gradual. When sampling dates were averaged for the entire season, there was essentially no difference in leaf content between Manchar and Sac. Leaf sheath comprised about 20% of the total plant dry matter at the June 1 sampling date and remained near 15% throughout the season. Differences between the bromegrass varieties were minor.

Although Sac was the later maturing variety, stem content was 8% greater than Manchar when averaged over sampling dates. Stem content increased most markedly during the heading and flowering stages of maturity and increased more gradually during the remainder of the season. It is readily apparent that the stem content of grasses is the most important fraction affecting digestibility later

TABLE 12. DRY MATTER CONTRIBUTION OF PLANT FRACTIONS TO THE TOTAL PLANT DRY MATTER OF BROMEGRASS VARIETIES

Date	Leaf		Sheath		Stem		Seedhead	
	Manchar %	Sac %	Manchar %	Sac %	Manchar %	Sac %	Manchar %	Sac %
6-1	68.5	66.4	18.4	21.6	13.1	11.9	--	--
6-10	52.1	52.8	18.4	14.9	20.9	16.1	8.5	12.2
6-19	29.7	33.5	28.4	15.9	24.1	30.8	17.8	19.7
6-28	25.6	23.2	15.7	15.9	35.9	34.2	22.5	26.7
7-7	21.1	20.2	13.0	13.6	37.2	41.1	28.6	25.1
7-16	18.5	20.0	13.1	12.9	43.4	45.9	25.0	21.1
7-25	13.5	19.7	13.2	14.9	41.4	50.0	31.9	15.4
8-3	18.1	18.6	13.3	15.6	43.8	49.5	24.8	16.3
8-12	14.6	16.8	14.6	15.9	49.9	56.7	20.9	10.6
$\bar{x}$	29.1	30.1	16.5	16.2	34.4	37.4	20.0	16.4

in the season as it accounts for about one-half of the plant dry matter.

The seedhead contributes a variable percentage of the plant dry matter as shown in table 12. As heading and seed development occurred, the seedhead increased gradually followed by a variable decline associated with shattering of the seed. Differences between varieties in the amount of dry matter accounted for by the seedhead were variable although the seasonal average showed Manchar to contain more than Sac.

Cellulose and acid-detergent fiber (ADF) content of bromegrass plant fractions are presented in table 13. Significance levels between varieties within species and between plant fractions are presented in table 14. Leaves and seedheads of Manchar bromegrass contained significantly ($P < .01$) less cellulose than sheath or stem fractions. Sheaths contained significantly ($P < .01$) more cellulose than leaves or seedheads but less than stems. Means for Manchar plant fractions were leaf, 25.1%; sheath, 36.7%; stem, 38.2%; seedhead, 25.8% and intact plant, 29.4%. Sac bromegrass leaves were lowest in cellulose content ($P < .01$) followed by seedheads, sheaths and stems. All fractions of Sac differed significantly ($P < .01$) from one another in cellulose content.

Analysis of variance for varietal differences showed that leaves and sheaths of Manchar contained more ($P < .01$) cellulose than the same fractions of Sac, while seedheads of Manchar were significantly ($P < .01$) lower than those of Sac.

TABLE 13. CELLULOSE AND ACID-DETERGENT FIBER CONTENT OF BROMEGRASS PLANT FRACTIONS

Date	Intact		Leaf		Sheath		Stem		Seedhead	
	M[1] %	S %	M %	S %	M %	S %	M %	S %	M %	S %
					Cellulose Content					
6-1	21.9	23.8	21.1	17.4	28.6	28.9	24.6	26.6	--	--
6-10	23.9	25.7	20.2	18.4	31.2	29.9	32.8	30.9	23.9	24.5
6-19	27.3	28.7	22.3	24.8	33.1	35.1	33.8	39.8	22.8	30.6
6-28	27.6	29.4	21.2	24.8	34.9	37.8	38.6	42.6	25.9	26.6
7-7	31.5	31.1	23.4	24.1	36.1	35.3	40.2	39.9	24.1	25.3
7-16	28.2	28.9	24.2	23.3	36.4	37.3	39.2	38.7	28.3	25.1
7-25	32.4	29.7	32.6	26.6	39.3	36.4	40.9	37.1	27.2	26.8
8-3	30.3	27.1	27.7	23.2	42.7	34.8	40.0	37.4	27.5	28.8
8-12	33.7	28.4	29.4	25.6	39.9	37.8	40.2	39.8	26.4	31.1
					Acid-Detergent Fiber					
6-1	23.1	21.9	21.8	22.0	28.8	30.7	24.5	25.9	--	--
6-10	25.1	25.7	22.5	24.7	32.3	33.1	32.4	28.2	25.4	25.4
6-19	30.4	28.5	25.8	25.1	35.9	37.7	35.8	39.3	26.3	29.4
6-28	33.2	32.2	27.7	26.9	36.3	38.4	40.4	44.6	28.5	30.8
7-7	32.6	32.7	28.8	27.5	38.1	39.7	43.1	42.6	29.2	30.4
7-16	32.4	33.7	31.4	36.5	40.0	43.6	42.0	45.7	33.9	34.9
7-25	34.2	36.5	34.7	35.8	40.1	42.1	42.4	44.7	32.4	37.4
8-3	33.8	34.8	36.1	34.6	42.9	41.3	44.4	44.9	33.4	38.8
8-12	36.5	36.3	36.9	36.1	42.5	44.3	47.9	45.0	36.2	39.5

[1] M = Manchar bromegrass, S = Sac bromegrass.

TABLE 14. MEAN SEASONAL CELLULOSE AND ACID-DETERGENT FIBER CONTENT OF PLANT FRACTIONS

Variety[1]	Intact	Leaf	Sheath	Stem	Seedhead
		Percent Cellulose Content			
Manchar	29.4[a]	25.1[b],2	36.7[c],2	38.2[d]	25.8[b],2
Sac	28.6[a]	23.8[b]	35.6[c]	38.3[d]	27.4[e]
Greenar	32.2[a],2	27.5[b],2	34.6[c]	38.5[d],2	27.3[b]
Oahe	33.7[a]	26.9[b]	34.5[c]	39.5[d]	27.7[e]
Siberian	32.9[a],2	26.6[b]	36.1[c]	40.8[d],2	28.3[e],3
Nordan	31.9[a]	26.6[b]	36.4[c]	37.9[d]	27.7[e]
		Percent Acid-Detergent Fiber			
Manchar	32.3[a]	30.5[b],3	38.5[c],2	41.0[d],2	30.7[b],2
Sac	32.6[a]	30.9[b]	40.0[c]	41.9[d]	33.3[e]
Greenar	38.1[a],2	34.1[b],2	39.1[c]	44.2[d]	32.0[e],2
Oahe	39.1[a]	32.6[b]	39.3[a]	44.1[c]	30.9[e]
Siberian	37.7[a],2	32.6[b],2	40.3[c],2	44.4[d],2	31.7[e],2
Nordan	35.6[a]	32.1[b]	39.2[c]	41.0[d]	28.9[e]

[a,b,c,d,e] Means in a row bearing a different superscript differ significantly ($P < .01$).

[1] Manchar and Sac - June 10 to August 15.
Greenar and Oahe - June 19 to August 15.
Siberian and Nordan - June 10 to August 15.

[2] Means in a pair differ at the 1% level.

[3] Means in a pair differ at the 5% level.

ADF content of plant fractions of bromegrass varieties followed the same trends as cellulose content. Leaves and seedheads of Manchar did not differ in ADF content but both of these fractions contained less ($P<.01$) ADF than sheaths and stems. Stems contained significantly ($P<.01$) more ADF than any other fraction of Manchar. All plant fractions of Sac bromegrass differed significantly ($P<.01$) from one another in ADF content. Leaves contained the least ADF followed by seedheads, sheaths and stems while the intact plant was intermediate between leaves and seedheads. Varietal differences between fractions showed that sheaths, stems and seedheads of Manchar contained significantly ($P<.01$) less ADF than similar fractions of Sac, while leaves of Sac contained more ($P<.05$) ADF than those of Manchar. ADF content of the intact plants did not differ significantly.

As shown in table 13 cellulose and ADF content of all plant fractions increased as plants matured. It was also apparent that leaves increased gradually in cellulose and ADF while the other fractions increased rapidly during the heading and flowering stages of maturity. Cellulose and ADF content of seedheads was variable for both Manchar and Sac bromegrass. Apparently this is due to the changes which occur in this fraction with maturity. The development of the seed, followed by shattering, could account for the variability shown. In general cellulose content was more variable than ADF content.

Acid-detergent lignin (ADL) and IVDMD values of bromegrass plant fractions are shown in table 15. Analysis of variance

TABLE 15. ACID-DETERGENT LIGNIN CONTENT AND IN VITRO DRY MATTER DIGESTIBILITY OF BROMEGRASS PLANT FRACTIONS

Date	Intact		Leaf		Sheath		Stem		Seedhead	
	M[1] %	S %	M %	S %	M %	S %	M %	S %	M %	S %
					Acid-Detergent Lignin					
6-1	3.9	3.5	3.5	3.8	3.4	3.9	2.5	2.6	--	--
6-10	3.4	3.9	3.4	3.2	4.3	4.5	3.5	3.1	4.4	3.9
6-19	5.1	6.4	4.4	4.9	5.2	5.9	4.9	5.2	6.2	7.9
6-28	5.3	6.6	5.0	7.2	6.3	7.3	7.2	7.7	6.7	7.3
7-7	6.2	6.6	8.2	7.4	7.0	7.8	8.8	7.6	8.6	6.8
7-16	7.4	7.3	9.7	8.7	7.5	6.4	8.1	7.2	8.1	6.8
7-25	8.3	6.3	8.9	8.6	8.1	6.9	8.5	7.7	8.7	7.5
8-3	8.5	6.5	7.8	8.9	8.2	6.9	9.2	8.0	7.7	7.2
8-12	9.9	5.8	9.7	7.9	8.6	7.0	8.9	7.5	8.3	6.4
					In Vitro Dry Matter Digestibility					
6-1	77.3	75.8	77.8	78.3	73.1	73.2	78.5	78.7	--	--
6-10	74.7	75.5	76.5	76.2	69.0	71.1	76.2	80.8	74.4	75.7
6-19	66.1	69.0	72.7	73.1	62.2	60.9	64.2	62.4	64.1	63.5
6-28	65.2	65.2	70.8	68.9	56.5	50.8	47.4	46.7	61.1	53.7
7-7	61.6	62.7	70.7	66.5	54.3	55.2	40.8	47.2	58.4	57.8
7-16	64.3	59.7	63.1	59.3	53.9	49.4	44.7	40.6	46.8	53.7
7-25	59.2	63.7	59.3	64.3	51.6	51.0	40.2	46.8	46.2	43.7
8-3	56.1	61.7	57.6	61.1	49.2	49.7	40.5	42.2	52.1	38.3
8-12	54.0	60.3	58.9	54.6	46.3	46.2	40.7	46.0	54.5	40.5

[1] M = Manchar bromegrass, S = Sac bromegrass.

indicating significance is shown in table 16. Sheaths of Manchar bromegrass contained significantly less ($P<.01$) ADL than stems and seedheads and also contained less ADL than leaves, although this difference was not significant. Leaves of Sac bromegrass contained significantly more ADL than sheath, stem or seedhead fractions. Manchar bromegrass intact plants, stems and seedheads contained significantly ($P<.01$) more ADL than similar fractions of Sac.

In all fractions as well as the intact plant ADL increased with later sampling date. ADL values were about 2.5 to 3.0% in early June and increased to 10% by mid-August. ADL values for leaves were considerably higher than expected. In some cases the leaves contained more lignin than sheaths which resembled stems in chemical composition. These high lignin values for leaves might be explained by the high protein content of this tissue which could increase ADL values if the extraction of protein nitrogen was incomplete. The variability of the ADL values cannot be explained. It is known that high drying temperatures can increase ADL values (Van Soest, 1965a); however, the samples used in this study were dried at low temperatures.

IVDMD of plant fractions of bromegrass varieties reflected chemical composition rather closely. Stems of both Manchar and Sac were significantly ($P<.01$) less digestible than any other fraction while leaves of these varieties were the most highly digestible of any fraction (table 16). Digestibility of leaf sheaths and seedheads was intermediate between that of leaves and stems. The intact plant and stem fractions of Manchar were significantly ($P<.01$) less

TABLE 16. MEAN SEASONAL ACID-DETERGENT LIGNIN CONTENT AND IN VITRO DRY MATTER DIGESTIBILITY OF PLANT FRACTIONS

Variety[1]	Intact	Leaf	Sheath	Stem	Seedhead
		Percent Acid-Detergent Lignin			
Manchar	$6.8^{a,2}$	7.1^{bc}	6.9^{ab}	$7.4^{c,2}$	$7.3^{c,2}$
Sac	6.2^{a}	7.1^{b}	6.6^{c}	6.8^{c}	6.7^{c}
Greenar	$6.1^{ab,2}$	$6.4^{b,3}$	5.9^{ab}	7.0^{c}	$5.9^{a,2}$
Oahe	6.6^{a}	6.0^{b}	5.9^{b}	6.7^{a}	4.7^{c}
Siberian	6.9^{a}	7.8^{b}	6.9^{a}	$8.2^{c,3}$	6.7^{d}
Nordan	6.8^{a}	7.4^{b}	6.4^{c}	6.5^{c}	6.4^{c}
		Percent In Vitro Dry Matter Digestibility			
Manchar	$62.7^{a,2}$	$66.2^{b,3}$	$55.4^{c,3}$	$49.4^{d,2}$	$57.2^{e,2}$
Sac	64.7^{a}	65.5^{a}	54.3^{b}	51.6^{c}	53.3^{b}
Greenar	$54.9^{a,2}$	$62.9^{b,2}$	$54.2^{a,3}$	$45.9^{c,2}$	$57.3^{d,2}$
Oahe	52.1^{a}	60.9^{b}	52.5^{a}	44.9^{c}	61.7^{b}
Siberian	$56.5^{a,2}$	$56.7^{a,2}$	$53.5^{b,2}$	$49.6^{c,2}$	$61.2^{d,3}$
Nordan	60.9^{ad}	62.3^{a}	55.5^{b}	52.7^{c}	60.2^{d}

^{a,b,c,d,e} Means in a row bearing a different superscript differ significantly ($P<.01$).

[1] Manchar and Sac - June 10 to August 15.
Greenar and Oahe - June 19 to August 15.
Siberian and Nordan - June 10 to August 15.

[2] Means in a pair differ at the 1% level.

[3] Means in a pair differ at the 5% level.

digestible than those of Sac. Leaves and seedheads of Sac were significantly ($P < .05$ and $P < .01$, respectively) less digestible than similar fractions of Manchar.

As shown in table 15 IVDMD values for leaves and stems were nearly identical on June 1 and June 10. After this date, or about the time of heading, IVDMD of stems decreased markedly while leaves declined slowly. The rapid decline in IVDMD of stem tissue along with its rapid increase during heading and flowering emphasizes the importance of harvesting early to obtain forage of high quality. The low digestibility of grasses late in the season is primarily due to the low digestibility of the stem and the large amount of it present. As shown earlier in table 12 the stem may account for 50 to 55% of the total plant dry matter of Manchar and Sac bromegrass. In addition to the low digestibility of stem tissue, the coarseness of the stem may decrease consumption late in the season.

IVDMD of sheath tissue declines less rapidly than that of stem tissue but also reaches a lower level than leaf digestibility. Seedheads also decline quite rapidly in IVDMD although the decline is not as rapid as that of sheaths and stems. Differences between varieties in IVDMD for bromegrass were relatively small although significant. Although leaves, sheaths and seedheads of Manchar bromegrass were more mature than those of Sac, they were slightly more digestible. This may indicate that Manchar would be more digestible than Sac when both are sampled at the same stage of maturity and this was shown in the *in vivo* digestion trial reported earlier.

Intermediate Wheatgrass Varieties. Dry matter contributed by each plant fraction to the total plant dry matter of intermediate wheatgrass varieties is shown in table 17. Greenar and Oahe intermediate reached the heading stage of maturity at the same date so that differences present between these varieties were probably due to inherent characteristics.

Leaf content was higher and stem content lower for intermediate wheatgrass varieties than for bromegrass or crested wheatgrass at the June 1 sampling date. These differences were due to maturity since intermediate wheatgrass was about two weeks later. Leaf content decreased rapidly during head emergence and declined steadily thereafter with very little difference between varieties. Sheath tissue accounted for about 32% of the plant dry matter on June 10 and decreased from that point on.

Stems of the intermediate wheatgrass varieties increased at the greatest rate during the heading stage and increased steadily thereafter. Differences between varieties at a given date were small and variable and are of little importance. Seedheads increased through mid-July and remained at this level through early August. The results of the plant separation data indicated that these two varieties are similar in growth patterns and morphological characteristics.

Cellulose and ADF content of Greenar and Oahe plant fractions is shown in table 18 and the statistical analysis of the data is presented in table 14. As shown in table 18 both cellulose and ADF content of plant fractions increased with later sampling date and the

TABLE 17. DRY MATTER CONTRIBUTION OF PLANT FRACTIONS TO THE TOTAL PLANT DRY MATTER OF INTERMEDIATE WHEATGRASS VARIETIES

	Leaf		Sheath		Stem		Seedhead	
Date	Greenar %	Oahe %	Greenar %	Oahe %	Greenar %	Oahe %	Greenar %	Oahe %
6-1	71.6	70.8	20.1	22.3	8.3	6.9	--	--
6-10	50.7	56.1	34.5	30.7	14.9	13.2	--	--
6-19	39.6	32.8	29.7	27.4	23.6	27.1	7.1	12.7
6-28	25.3	25.4	21.6	19.6	38.2	39.5	14.9	15.6
7-7	20.7	20.8	20.4	17.6	42.5	46.3	16.5	15.4
7-16	18.1	17.2	20.1	18.0	43.9	46.9	17.9	17.8
7-25	12.0	14.4	21.2	16.9	46.8	48.7	19.9	19.9
8-3	13.2	14.2	19.5	17.9	53.6	46.5	13.8	21.4
8-12	10.1	11.8	15.1	21.4	55.8	56.2	19.1	10.7
$\bar{x}$	29.0	29.3	22.4	21.3	36.4	36.8	12.2	12.6

TABLE 18. CELLULOSE AND ACID-DETERGENT FIBER CONTENT OF INTERMEDIATE WHEATGRASS PLANT FRACTIONS

Date	Intact		Leaf		Sheath		Stem		Seedhead	
	G[1] %	O %	G %	O %	G %	O %	G %	O %	G %	O %
					Cellulose Content					
6-1	23.5	23.2	21.9	21.7	28.6	28.4	24.6	24.0	--	--
6-10	23.9	23.9	23.9	20.8	30.2	27.4	31.8	27.4	--	--
6-19	29.4	29.8	25.8	21.7	33.1	32.7	34.6	36.0	25.7	30.7
6-28	32.5	33.0	24.1	25.4	35.3	34.4	39.9	40.6	29.5	30.7
7-7	30.2	35.9	24.2	24.2	37.1	35.2	39.3	39.3	28.0	27.6
7-16	33.3	33.9	26.4	25.7	32.6	34.2	39.7	39.6	26.5	24.5
7-25	32.2	32.7	29.4	29.6	34.5	34.3	39.2	40.9	26.3	23.7
8-3	33.3	33.6	30.3	30.0	35.6	35.5	39.0	40.0	28.9	26.5
8-12	34.5	36.7	32.1	32.1	34.1	34.9	37.5	40.1	25.9	30.4
					Acid-Detergent Fiber					
6-1	24.7	24.6	23.7	23.3	29.2	29.9	23.7	22.0	--	--
6-10	26.0	26.5	26.5	23.9	30.9	28.6	30.7	26.8	--	--
6-19	30.2	33.4	26.9	25.3	34.3	35.4	36.7	35.8	24.3	26.6
6-28	36.9	37.1	28.3	27.3	38.6	38.2	44.9	43.5	36.9	35.3
7-7	38.7	40.3	32.3	28.2	38.5	38.9	46.4	45.1	35.4	31.1
7-16	40.8	40.7	34.4	32.3	38.7	38.5	45.0	45.1	31.6	31.1
7-25	39.3	38.8	38.7	36.5	41.1	40.7	45.6	45.8	31.4	26.1
8-3	39.8	40.8	38.9	38.3	40.8	41.9	44.9	47.7	33.8	29.5
8-12	41.1	42.7	38.9	40.4	41.9	41.3	45.6	45.7	30.9	36.4

[1] G = Greenar and O = Oahe intermediate wheatgrass.

increase in ADF was greater than for cellulose. Leaves of Greenar contained significantly ($P<.01$) less cellulose than the intact plant, leaf sheath or stem fractions. Sheaths contained significantly less cellulose than stems and about 20% less than leaves. Oahe plant fractions all differed significantly from one another in cellulose content. Leaves contained the least cellulose followed by the seed-head, sheath and stem fractions.

Varietal differences which were significant included cellulose content of the intact plant, leaf and stem fractions. Intact plants and stems of Oahe contained significantly ($P<.01$) more cellulose than Greenar, while leaves of Oahe contained less cellulose than those of Greenar. These differences are small and unimportant when these grasses are harvested at or before the heading stage of maturity.

ADF values followed a trend similar to those of cellulose for fractions of Greenar and Oahe. In both of these varieties the seedhead was lowest in ADF followed by leaves, sheaths and stems. Plant fractions of Greenar all differed significantly ($P<.01$) from one another while intact plants and sheaths of Oahe were similar in ADF content. As with cellulose, stems contained approximately 25% more ADF than leaves and about 10% more than sheaths. The observation that stems of these varieties contain 44% ADF may explain the low digesti-bility obtained in the *in vivo* trial as well as the poorer consumption of this forage by the lambs.

Intact plants of Oahe contained significantly ($P<.01$) more cellulose than Greenar while leaves and seedheads of Greenar contained

significantly ($P<.01$) more ADF than the same fractions of Oahe. Although these differences were small, they no doubt affected the digestibility of the particular fraction and the intact plant.

From the data presented in table 18 it is apparent that the ADF values are approximately 6 to 8 percentage units larger than cellulose values. These higher values are due to the inclusion of lignin in the ADF analysis. The ADF analysis follows maturation more closely than cellulose as evidenced by the higher correlations with digestibility. Cellulose increases from 28 to 35% while ADF increases from 23% in June to 45% in mid-August.

Acid-detergent lignin (ADL) and in vitro dry matter digestibility (IVDMD) of intermediate wheatgrass plant fractions are shown in table 19. ADL increased with later sampling date for all fractions as well as the intact plant. The increase with later sampling date was greater for stems than the other fractions, while leaves, sheaths and seedheads increased at about the same rate. The relatively high ADL content of leaves was not expected; however, the lower leaves of these plants were dead and weathered which could increase the ADL content more than would be expected.

Seasonal means shown in table 16 indicated that intact plants, sheaths and seedheads of Greenar did not differ significantly in ADL content. Stems of Greenar contained significantly more ADL than any other fraction. Stems and intact plants of Oahe did not differ significantly in ADL content but contained significantly ($P<.01$) more than leaves, sheaths and seedheads. Seedheads of Oahe contained less

TABLE 19. ACID-DETERGENT LIGNIN CONTENT AND IN VITRO DRY MATTER DIGESTIBILITY OF INTERMEDIATE WHEATGRASS PLANT FRACTIONS

	Intact		Leaf		Sheath		Stem		Seedhead	
Date	G[1] %	O %	G %	O %	G %	O %	G %	O %	G %	O %
					Acid-Detergent Lignin					
6-1	3.7	3.2	3.0	3.3	3.8	3.2	3.8	4.9	--	--
6-10	3.2	4.2	4.4	4.5	4.1	3.7	3.0	3.8	--	--
6-19	4.3	5.6	5.1	4.1	4.8	4.5	4.9	4.6	3.0	2.8
6-28	5.7	5.3	5.6	4.4	5.4	4.8	5.8	5.3	7.9	4.3
7-7	5.9	6.7	6.6	5.2	5.7	6.1	8.1	8.1	6.7	4.5
7-16	7.1	6.7	7.2	5.3	5.9	5.0	8.2	6.9	5.6	4.8
7-25	6.7	6.3	7.3	6.4	7.0	7.1	7.5	7.1	6.4	4.5
8-3	6.9	7.8	6.7	6.8	6.4	6.8	7.2	7.9	5.4	4.9
8-12	6.0	7.7	6.2	6.9	6.7	7.1	7.3	7.2	6.2	6.7
					In Vitro Dry Matter Digestibility					
6-1	66.3	70.4	69.3	69.3	67.2	70.8	73.3	75.6	--	--
6-10	71.6	68.0	72.0	70.1	69.4	67.1	80.1	72.2	--	--
6-19	68.5	59.6	68.9	67.9	58.4	60.7	60.4	60.2	70.7	73.5
6-28	55.5	55.7	64.4	66.6	53.6	53.8	52.3	49.4	52.9	58.8
7-7	55.7	49.5	65.3	67.3	59.5	50.8	40.8	44.1	55.8	60.6
7-16	50.6	50.3	61.1	65.9	49.5	54.8	43.5	40.3	58.1	60.1
7-25	50.3	48.9	60.5	52.6	50.2	50.1	37.1	40.9	53.8	63.3
8-3	53.6	50.5	59.9	49.5	53.9	47.4	49.9	34.6	51.1	63.8
8-12	50.8	50.3	60.4	56.9	54.2	49.5	37.7	44.8	58.5	51.9

[1] G = Greenar and O = Oahe intermediate wheatgrass.

ADL than any of the other fractions. A comparison of varietal means showed that leaves and seedheads of Greenar contained significantly more ADL than those of Oahe ($P < .05$ and $P < .01$, respectively). However, intact plants of Oahe contained significantly ($P < .01$) more ADL than Greenar intact plants, 6.6% compared to 6.1%. From the data shown in table 19, ADL content of Oahe intact plants increased more rapidly later in the season when compared to Greenar.

IVDMD values shown in table 19 for the intermediate wheatgrass varieties indicate that stems of these varieties were more digestible than leaves at the June 1 and 10 sampling dates. However, the decline in stem digestibility was much more rapid than that of leaves so that on June 19 leaves were about 12% more digestible than stems. The rapid decline in stem digestibility was associated with stem elongation prior to and during heading of the varieties. Leaf sheaths appeared lowest in IVDMD of any fraction on June 1. This tissue is probably the most mature part of the plant at this time and serves as the structural material before stem elongation begins. The leaf sheath resembles the stem in content of fibrous components and lignin; however, IVDMD of the sheath does not decline to as low a level as that of stems. The seedhead was highly digestible during early stages of heading but declined rapidly. From table 19 it can also be seen that digestibility of the seedhead exhibited considerable variability. This variation might be explained by development of the endosperm followed by seed shattering later in the growing season. The variability in IVDMD of the seedhead followed quite closely the variation in ADF content of this fraction.

Plant fraction means shown in table 16 revealed that leaves of Greenar were more digestible than any other fraction. Seedheads were significantly ($P<.01$) more digestible than sheaths and stems, while digestibility of sheaths and intact plants did not differ significantly. Stems were lower in IVDMD than any of the other fractions, a reflection of ADF and ADL content which were highest for these fractions. Leaves and seedheads of Oahe were significantly ($P<.01$) more digestible than sheaths and stems. Stems were lowest in IVDMD followed by leaf sheaths. IVDMD of intact plants of Oahe was similar to that of sheaths and intermediate between that of leaves and stems. Although analysis of variance was conducted on a within species basis, it is apparent that stems of the intermediate wheatgrass varieties were considerably lower in IVDMD than the other species even though these varieties were two weeks later in reaching the heading stage of maturity.

Intact plants, leaves, sheaths and stems of Greenar were all significantly ($P<.01$ or $P<.05$) more digestible than similar fractions of Oahe. Seedheads of Oahe were significantly ($P<.01$) more digestible than those of Greenar. The higher ADF and ADL contents of Greenar seedheads were reflected in lower IVDMD values. The differences present in chemical composition and IVDMD between varieties of intermediate wheatgrass were due to morphological characteristics since both Greenar and Oahe reached the heading stage of maturity at the same date. The differences were not explainable by the amount of dry matter contributed by individual plant fractions to the total plant

dry matter since very little difference was present between varieties in this respect.

Crested Wheatgrass Varieties. Dry matter contribution of each plant fraction to the total plant dry matter of crested wheatgrass varieties is shown in table 20. The lower percentage of leaf content and higher percentage of sheath and stem tissue on June 1 are indicative of the earlier maturity of these varieties when compared to bromegrass and intermediate wheatgrass. Leaf content of Nordan declined more rapidly than that of Siberian early in June; however, the seasonal mean was nearly identical for the two varieties. The importance of early harvesting of these varieties is emphasized by the fact that leaves account for only about 10% of the plant dry matter in mid-July. Sheath content of the intermediate wheatgrass varieties declined until June 28 and then leveled off with little difference between varieties. As with the other species, stem tissue increased most rapidly during the heading and seed development stages of maturity. Nordan appeared to increase more rapidly than Siberian. This could be related to maturity differences since Nordan was four days earlier in reaching the heading stage. The seasonal average showed that Nordan contained 5% more stem tissue than Siberian. At the August 12 sampling date these varieties contained 65% stem tissue, indeed an indication that these varieties should be harvested long before this date to obtain high quality forage. The amount of dry matter accounted for by seedheads increased through July 16 and

TABLE 20. DRY MATTER CONTRIBUTION OF PLANT FRACTIONS TO THE TOTAL PLANT DRY MATTER OF CRESTED WHEATGRASS VARIETIES

	Leaf		Sheath		Stem		Seedhead	
Date	Siberian %	Nordan %	Siberian %	Nordan %	Siberian %	Nordan %	Siberian %	Nordan %
6-1	55.1	51.2	30.3	30.6	14.6	18.3	--	--
6-10	40.8	29.9	21.2	23.7	31.0	32.7	6.9	13.6
6-19	23.8	21.9	16.7	15.9	41.9	40.0	17.5	21.9
6-28	16.7	14.1	13.3	10.4	45.5	52.9	24.5	22.6
7-7	14.0	16.3	11.9	12.4	52.3	50.7	21.8	20.6
7-16	10.4	13.4	12.3	13.2	48.4	47.6	28.9	25.7
7-25	6.2	8.9	11.3	7.5	56.6	61.3	25.9	22.2
8-3	10.9	9.4	13.7	11.4	58.9	64.3	16.5	14.9
8-12	10.7	8.3	14.3	12.9	64.4	67.9	10.5	10.8
$\bar{x}$	20.9	19.3	16.1	15.4	45.9	48.4	16.9	16.9

decreased thereafter. Both varieties contained the same amount of seedhead tissue when all sampling dates were averaged.

Cellulose and ADF values for Siberian and Nordan crested wheatgrass plant fractions are shown in table 21. Cellulose content increased significantly ($P<.01$) with later sampling date for all fractions as well as the intact plant. Leaves of both Siberian and Nordan remained essentially constant until July 16 at which time cellulose content increased rapidly. Leaf sheaths were considerably higher in cellulose content than leaves throughout the study and contained nearly as much cellulose as stems early in the season. Seedhead fractions exhibited considerable variability in cellulose content with later sampling date.

Fraction means shown in table 14 indicated that all fractions differed significantly ($P<.01$) in cellulose content for both varieties. Leaves contained the least cellulose followed by the seedhead, intact plant, sheath and stem fractions in order of increasing cellulose content. Leaves contained 32% less cellulose than stems and 27% less than leaf sheaths.

ADF values shown in table 21 followed trends similar to those of cellulose content. Leaves of the two varieties increased more rapidly in ADF content than cellulose and reached a relatively high level in August. Leaves contained nearly as much ADF as sheaths and stems in August. The earlier maturity would allow greater weathering and leaching losses to occur in the crested wheatgrass varieties when compared to bromegrass and intermediate wheatgrass varieties. Although

TABLE 21. CELLULOSE AND ACID-DETERGENT FIBER CONTENT OF CRESTED WHEATGRASS PLANT FRACTIONS

Date	Intact		Leaf		Sheath		Stem		Seedhead	
	S[1] %	N %	S %	N %	S %	N %	S %	N %	S %	N %
					Cellulose Content					
6-1	25.1	24.5	22.9	24.7	29.7	27.9	31.9	29.3	--	--
6-10	28.2	28.1	23.8	23.2	33.7	33.7	34.2	36.1	27.8	26.9
6-19	33.3	30.4	22.3	22.4	35.8	38.2	41.4	34.3	34.6	30.5
6-28	32.2	29.6	21.9	23.7	35.7	35.7	44.2	41.5	26.6	28.2
7-7	34.2	33.6	22.9	24.3	34.8	34.2	40.7	39.1	27.4	26.3
7-16	34.6	31.7	27.7	27.6	34.5	36.5	42.4	39.2	23.9	24.0
7-25	33.0	32.5	29.8	27.6	38.2	36.7	44.2	36.6	23.7	23.1
8-3	33.8	34.2	31.7	31.2	37.9	38.1	40.9	38.4	27.7	28.9
8-12	33.9	34.9	32.3	32.9	37.8	38.4	38.5	37.8	34.3	33.7
					Acid-Detergent Fiber					
6-1	26.2	22.5	23.3	22.4	28.5	27.1	28.8	29.5	--	--
6-10	28.5	27.9	23.8	23.5	32.9	33.7	33.4	35.3	23.4	24.9
6-19	34.7	30.9	24.7	26.5	37.7	41.2	41.8	39.8	32.4	23.1
6-28	36.9	36.6	27.1	30.1	38.9	39.8	47.1	45.3	31.4	30.4
7-7	39.6	38.3	29.1	29.2	39.5	36.1	46.3	41.8	38.5	28.4
7-16	39.7	36.2	33.9	33.7	39.6	40.6	47.8	42.1	27.5	25.3
7-25	40.3	36.3	38.8	34.8	44.6	39.9	47.7	39.6	26.9	26.2
8-3	40.6	38.6	40.8	39.3	44.8	41.0	46.9	42.6	33.8	33.8
8-12	41.2	40.2	42.8	40.0	44.6	41.4	44.3	41.9	39.7	36.8

[1] S = Siberian and N = Nordan crested wheatgrass.

Nordan contained more stem tissue than Siberian, ADF content of Siberian stems increased more rapidly and reached a higher level than stems of Nordan. Siberian was also the later maturity variety which indicates that the higher ADF content of stems of this variety was due to inherent characteristics. Seedheads of crested wheatgrass varieties fluctuated in ADF content in a manner similar to cellulose content.

All fractions as well as the intact plants of Nordan and Siberian differed significantly ($P<.01$) in ADF content (table 14). In both varieties seedheads were lowest in ADF followed by leaves, intact plants, sheaths and stems in order of increasing fiber content. Leaves contained about 24% less ADF than stems. All fractions of Siberian contained significantly ($P<.01$) more ADF than fractions of Nordan with the largest difference occurring between stems of the two varieties.

ADL and IVDMD values for Siberian and Nordan are presented in table 22. ADL increased significantly ($P<.01$) with later sampling date for all plant fractions as well as the intact plant. Leaves of Siberian crested wheatgrass contained more ADL than any other fraction at the August sampling dates. Leaves of Nordan appeared to increase in ADL more rapidly and earlier in the season than leaves of Siberian but did not attain values as high as those of Siberian. At the June 1 sampling date stems were lowest in ADL but were similar to other fractions as maturity advanced. Seedheads were also variable in ADL content as was shown for cellulose and ADF content. Analysis of variance showed that stems of Siberian contained significantly ($P<.01$)

TABLE 22. ACID-DETERGENT LIGNIN CONTENT AND IN VITRO DIGESTIBILITY OF CRESTED WHEATGRASS PLANT FRACTIONS

Date	Intact		Leaf		Sheath		Stem		Seedhead	
	S[1] %	N %	S %	N %	S %	N %	S %	N %	S %	N %
					Acid-Detergent Lignin					
6-1	3.9	4.4	3.7	3.5	3.5	3.7	2.5	2.2	--	--
6-10	3.9	3.5	4.6	3.9	4.6	4.3	4.3	3.5	2.7	3.3
6-19	5.2	5.9	5.7	6.4	5.3	6.6	6.0	5.2	5.7	6.5
6-28	6.6	7.1	5.4	8.2	5.9	6.2	7.7	7.8	8.0	8.1
7-7	7.4	7.7	5.8	8.6	7.1	6.3	8.6	7.1	6.1	7.6
7-16	8.6	7.8	8.4	8.6	7.9	7.2	9.8	7.2	6.6	5.7
7-25	8.1	6.9	10.8	8.3	7.9	6.8	10.6	6.8	7.1	5.8
8-3	7.9	7.1	10.7	7.4	8.9	6.6	8.9	7.3	8.3	7.0
8-12	7.9	8.2	11.0	7.8	8.2	7.0	9.5	7.4	8.9	7.3
					In Vitro Dry Matter Digestibility					
6-1	76.3	84.2	77.5	78.2	80.3	75.7	85.9	80.3	--	--
6-10	74.8	74.5	76.2	77.1	63.2	49.3	68.0	69.5	76.2	65.3
6-19	58.7	65.9	75.1	73.5	58.1	59.1	62.9	58.6	66.0	67.2
6-28	58.8	64.5	70.1	71.8	53.7	62.8	52.1	58.1	65.1	60.3
7-7	56.5	57.9	67.5	59.8	54.4	59.6	43.7	50.3	57.5	62.2
7-16	50.5	61.0	57.2	55.1	56.4	56.2	44.4	50.1	65.2	64.7
7-25	54.8	60.0	40.5	50.1	48.6	52.2	39.3	45.6	61.8	59.9
8-3	47.9	52.6	32.6	47.5	50.8	51.9	40.9	44.7	54.2	52.4
8-12	49.9	50.8	34.7	53.8	42.6	52.4	45.6	45.0	43.7	49.4

[1] S = Siberian and N = Nordan crested wheatgrass.

more ADL than any other fraction (table 16). Leaves contained more ADL than sheaths and seedheads but less than stems. Seasonal averages also showed that leaves of Nordan contained significantly ($P<.01$) more ADL than any other fraction. This difference was in part due to the high ADL content of Nordan leaves in August. At these dates leaf tissue accounted for only 10% of the total plant dry matter. Sheaths, stems and seedheads of Nordan did not differ significantly in ADL content. The only significant difference between varieties was in ADL content of stems. Nordan crested wheatgrass stems contained significantly ($P<.05$) less ADL than stems of Siberian.

IVDMD values presented in table 22 show that stems and sheaths were higher in IVDMD than leaves of Siberian and Nordan crested wheatgrass early in the season. Digestibility of leaves declined slowly until July 7 after which time it declined rapidly. As shown leaves of Siberian were of very low digestibility in late July and early August, a reflection of their high lignin content at these sampling dates. IVDMD values for sheath fractions declined at a steady rate after a rapid decline in early June. Stems of both Siberian and Nordan declined rapidly during the preheading and heading stages of maturity. IVDMD of stems did not decrease to a lower level than leaves and sheaths of Siberian crested wheatgrass. Digestibility of the seedhead fraction reflected the variability in chemical composition as IVDMD values were also variable.

Analysis of variance of IVDMD values showed that stems of Siberian were significantly ($P<.01$) less digestible than any other

fraction (table 16). Leaf sheaths of Siberian were more ($P<.01$) digestible than stems but not leaves or seedheads. Leaves of Nordan were significantly ($P<.01$) more digestible than any other fraction. Sheath tissue IVDMD values were intermediate between stems and leaves.

Varietal means showed that intact plants, leaves, sheaths and stems of Nordan were all significantly ($P<.01$) more digestible than similar fractions of Siberian, the largest difference being IVDMD of leaves where Nordan leaves were approximately 9% more digestible. These differences indicate that Nordan crested wheatgrass is slightly superior to Siberian since Nordan was the earlier maturing variety. When these varieties were compared at equal stages of maturity by *in vivo* methods, Nordan was also slightly superior in digestibility.

Regardless of species, interactions which were significant in the plant separation study included the fraction by date, fraction by variety, date by variety and fraction by date by variety interactions. These interactions point out the fact that the varieties respond differently at different times during the growing season.

Correlation coefficients computed by using the values obtained for intact plants showed correlations between IVDMD and cellulose content, ADF, ADL, percent leaf and percent stem to be highly significant. Correlations were -.87, -.93, -.79, 0.79 and -.79, respectively. Correlation coefficients between ADF and percent leaf and percent stem were -.89 and 0.89 ($P<.01$), respectively. The highly significant correlation of -.94 between percent leaf and percent stem

indicates that these components vary nearly inversely during the growing season.

The results of the plant separation study are generally in agreement with previous work published in this area. In reviewing the literature little information was found concerning the fiber and lignin content of plant fractions. A study reported by Pigden and Heinrichs (1957) showed that lignin content was higher in stems (including sheath) than in leaves and that leaf percentage had a greater effect on lignin content than any other factor studied. Jarrige (1960) also reported that stems contained a greater proportion of membranes than leaves. Cellulose and lignin content of stems and sheaths of grasses rose rapidly with advancing maturity while leaves increased at a less rapid rate. The results obtained in these studies are similar to those reported in the present study. In general, leaves contained less cellulose and ADF than any other fraction. Seedheads contained more cellulose and ADF than leaves but less than sheaths while stems were highest in these components. ADL content was usually highest in stems followed by sheaths, seedheads and leaves in decreasing order. In some instances leaves contained nearly as much or more lignin than stems. This appeared only in very mature leaves which were weathered to a considerable extent. Little information was available on varietal differences in lignin or fiber content; however, a study by Sosluski et al. (1960) comparing the lignin content of varieties of bromegrass, intermediate and crested wheatgrass showed that small differences were

present between varieties. The differences were variable between years and locations and no trends were established.

The contribution of various fractions to the total plant dry matter has been studied by Minson et al. (1960a) and Mowat et al. (1965c). These workers found that percentage leaf decreased rapidly prior to head emergence and then decreased more slowly. These findings are in agreement with work reported in this study. In general, leaf content decreased most rapidly during head emergence and flowering and declined more slowly after this period. Stem tissue increased most rapidly during heading and flowering stages and increased gradually but steadily after this time. Sheath content was highest in early harvests and declined slowly thereafter, usually comprising 15 to 20% of the plant dry matter. Seedheads were at a maximum shortly after heading and decreased from then on.

Several studies have been reported on the digestibility of various plant fractions. Work by Pritchard et al. (1963) and Terry and Tilley (1964) showed that digestibility of all fractions decreased as season advanced, but the decrease was greatest for stem tissue. The study of Terry and Tilley (1964) indicated that the leaf blade, the major fraction before head emergence, declined most slowly and stem tissue which predominated after head emergence declined most rapidly. Leaf sheath and seedhead fractions had intermediate values. These findings are in agreement with the work reported in this paper.

The IVDMD technique has also been used to study varietal and clonal differences by Mowat et al. (1965a, 1965b, 1965c) and Christie

and Mowat (1968). These workers found significant differences in IVDMD between varieties and clones when harvested at similar stages of maturity. Although the study reported here did not take maturity differences into account, significant differences were present between varieties of intermediate wheatgrass and crested wheatgrass. The varieties of intermediate were of similar maturity while Nordan crested wheatgrass was four days earlier in reaching the heading stage than Siberian. Intact plants and all fractions of Nordan except seedheads were more digestible than Siberian.

In summarizing the implications of this study, it can be said that the rapid increase in stem tissue associated with heading of grasses and the associated rapid decline in digestibility of stem tissue indicate the need for harvesting at or shortly after the heading stage of maturity. The results also indicate that the selection for stems of high digestibility by plant breeders may be more beneficial than selection for leafiness since leaves remain higher in digestibility for longer periods.

In addition, differences between varieties in chemical composition and IVDMD were small although significant in many cases. The differences shown to be significant are surely of minor importance when these grasses are harvested at the recommended stage of maturity.

Effects of Limited Soil Moisture on Chemical Composition and In Vitro Digestibility of Grasses

This portion of the study, although not a part of the original research proposal, became necessary due to the droughty conditions

which existed in 1967. During early May of the 1967 study it became apparent that soil moisture distribution in the experimental plots was not uniform. Soil samples were taken and it was determined that subsoil gravel deposits about 0.5 to 1.0 m. below the surface resulted in moisture losses in certain areas which caused unequal plant growth. These areas were not apparent during the 1966 study; however, late fall and winter precipitation in 1966 and early spring precipitation in 1967 was nearly 13 cm. below normal. Areas which were not underlaid by gravel deposits or where these deposits were more than 1.5 m. below the surface showed normal plant growth.

In addition, soil samples revealed that the shallowness to gravel resulted in a highly alkaline soil at the surface. This could result in a portion of the phosphorus being bound in the inorganic form which would have been unavailable to the plants. The ability of grasses to withstand drought is important to the plant breeder and the effect of drought on the chemical composition and digestibility is important to the animal nutritionist. With this in mind it was decided to sample both areas rather than using one area or the other.

Sampling dates were the same as used in the 1966 study; namely, every sixth day beginning May 23 and continuing through August 15. Preparation of samples prior to analysis was also similar to 1966. Maturity of the grasses grown on soil of normal moisture was approximately one week earlier than in 1966, while those grown on soils of below normal moisture matured at about the same date as in 1966.

Throughout the discussion the grasses will be referred to as being grown on normal or dry soils.

Plant height measurements taken on June 10 showed that plants on normal soil were 56, 30 and 50% taller, respectively, for bromegrass, intermediate and crested wheatgrass than those grown on dry soils. Yield data taken when each variety reached the 50% headed stage showed that yields averaged 3460 and 3900 kg. per hectare, respectively, for varieties of crested wheatgrass and bromegrass on normal soils. Yields on dry soils were 2110 and 2670 kg. per hectare, respectively, for crested wheatgrass and bromegrass. Intermediate wheatgrass varieties averaged 5115 kg. per hectare on both normal and dry soils.

Although growth of the intermediate varieties was retarded early in the season, this species apparently compensated for the retarded growth. This may be explained by the two week later maturity of intermediate wheatgrass when compared to the other species. The later maturity allowed this species to benefit more from rainfall in early June which was considerably above normal. Normal precipitation for the month of June was 10 cm., while rainfall during June, 1967, was 22.6 cm.

The samples grown on normal soils have been reported in the 1966 and 1967 comparison. Differences between varieties within a species were the same as reported in the above mentioned comparison, but they were of lower magnitude. In the following three tables varieties have been averaged and therefore will not be emphasized in the discussion.

Chemical composition and *in vitro* digestibility of bromegrass are shown in table 23. Regardless of soil moisture or species cellulose, ADF and ADL increased significantly while IVDMD and IVCD declined significantly ($P<.01$) with later sampling date. Cellulose content of bromegrass grown on dry soils was 3.7% lower than that of plants grown on normal soil when all sampling dates were averaged. As shown in table 23 there was essentially no difference in cellulose content during late July and August. ADF values followed a trend similar to that of cellulose in that ADF content of plants grown on normal and dry soils did not differ in late July and August. However, the seasonal mean was significantly different, 34.7% ADF for plants grown on normal soils and 32.9% for plants grown on dry soils. ADL content did not differ significantly between plants grown on either normal or dry soils. The study of Patton and Gieseker (1942) showed that grasses in a more arid region of Montana reached maximum lignin content earlier in the season. This was not found to be the case in this study. It would be expected that lignin content would follow a pattern similar to ADF; however, this did not occur. Denium (1966) also showed that under dry conditions crude fiber and water soluble carbohydrate content of grasses decreased.

In vitro digestibility data reflected the altered chemical composition of the grasses. IVDMD was significantly ($P<.01$) higher for bromegrass grown on soil of below normal moisture content when compared to plants grown on soils of normal moisture content. This difference was approximately 4%. Values were 59.9 and 62.4%,

TABLE 23. CHEMICAL COMPOSITION AND IN VITRO DIGESTIBILITY OF BROMEGRASS AS AFFECTED BY SOIL MOISTURE SUPPLY[1]

	Cellulose		ADF		ADL		IVDMD		IVCD	
	N[2]	D	N	D	N	D	N	D	N	D
Date	%	%	%	%	%	%	%	%	%	%
May 23	23.0	20.6	23.4	21.4	3.4	3.7	73.8	77.7	74.5	75.8
May 29	22.2	19.9	24.3	22.3	3.4	3.3	73.8	74.9	74.8	78.1
June 4	23.8	20.4	26.4	22.9	4.4	4.9	70.3	74.9	71.9	76.8
June 10	26.5	22.6	30.1	25.4	4.5	4.5	68.6	71.2	66.7	76.1
June 16	27.5	24.7	32.6	29.0	6.2	5.8	64.9	69.9	60.7	67.1
June 22	28.1	27.2	34.6	30.6	6.0	5.3	62.0	69.8	53.4	63.9
June 28	30.4	28.6	34.9	33.8	6.1	5.5	60.9	63.7	51.5	57.9
July 4	29.8	28.2	35.4	35.2	6.6	6.6	62.1	64.2	50.4	52.5
July 10	29.2	31.0	38.1	36.1	7.2	6.8	57.7	58.8	42.8	47.9
July 16	29.3	28.0	37.4	34.9	6.9	7.1	55.7	58.5	45.2	42.7
July 22	32.1	31.9	38.9	36.9	6.8	7.3	52.9	56.3	45.3	47.3
July 28	31.2	33.8	40.6	40.3	7.4	7.9	49.6	50.3	36.6	39.1
Aug. 3	33.7	34.4	39.8	40.0	7.1	7.3	49.5	49.8	37.7	39.4
Aug. 9	34.7	34.3	41.6	41.3	9.2	8.6	48.4	47.9	37.6	39.3
Aug. 15	34.5	34.9	42.5	42.6	9.6	8.9	48.3	48.1	47.3	46.7

[1] Average of two varieties.
[2] N = normal soil moisture, D = below normal soil moisture.

respectively, for bromegrass grown under normal and dry conditions. IVCD values were also significantly ($P<.01$) lower for bromegrass grown under normal soil moisture when compared to dry conditions. Means were 53.0 and 56.7%, respectively, a difference of 6.5%. As pointed out earlier, cellulose and ADF differed very little in late July and August and this was also reflected by the IVDMD values but not by the IVCD values. As was mentioned earlier, plants growing under dry conditions reached the heading stage of maturity about one week later than those on normal soils. It is doubtful that this factor alone accounted for the altered composition and *in vitro* digestibility since studies by Christie and Mowat (1968) have shown that later heading plants are less digestible than earlier maturing plants.

The below normal soil moisture levels appeared to affect chemical composition and *in vitro* digestibility of intermediate wheatgrass to a lesser extent than the other species (table 24). Cellulose content was significantly ($P<.01$) higher in plants grown on soil of normal moisture content; however, the difference was only 1.7%. Cellulose content increased significantly ($P<.01$) with later sampling date and was highest for plants on normal soils during August.

ADF values for intermediate wheatgrass also increased significantly ($P<.01$) with later sampling date and were significantly lower ($P<.01$) for plants grown under dry conditions. Mean ADF content was 34.2 and 33.3%, respectively, for plants from normal and dry soils. ADL values increased significantly ($P<.01$) with later sampling date

TABLE 24. CHEMICAL COMPOSITION AND IN VITRO DIGESTIBILITY OF INTERMEDIATE WHEATGRASS AS AFFECTED BY SOIL MOISTURE SUPPLY[1]

	Cellulose		ADF		ADL		IVDMD		IVCD	
Date	N[2] %	D %	N %	D %	N %	D %	N %	D %	N %	D %
May 23	22.3	21.7	23.9	22.6	3.3	4.5	76.2	78.1	74.5	74.3
May 29	21.9	20.9	24.2	23.3	3.8	4.0	74.9	74.0	79.3	79.8
June 4	21.6	23.7	25.3	24.6	4.5	4.6	73.3	72.1	73.8	77.6
June 10	26.1	25.4	28.4	26.3	4.7	4.5	70.6	72.3	75.2	81.0
June 16	27.5	26.4	30.9	29.9	4.8	5.9	69.9	71.8	70.7	73.9
June 22	29.7	27.7	33.3	30.9	5.1	4.9	66.9	70.1	69.1	69.6
June 28	32.7	32.5	36.1	35.4	4.9	4.6	68.8	68.5	68.7	65.8
July 4	31.7	29.6	37.1	35.5	5.5	5.8	69.2	67.9	62.1	60.0
July 10	30.9	29.9	38.3	37.2	6.8	6.8	59.8	61.3	59.5	53.7
July 16	30.9	29.2	37.5	35.9	6.6	6.5	60.4	63.4	56.2	57.5
July 22	30.7	29.9	37.1	37.2	6.2	6.3	60.1	61.5	51.3	52.6
July 28	33.3	32.6	38.5	38.3	6.3	6.8	58.1	58.2	56.2	53.6
Aug. 3	35.8	36.5	39.7	40.1	7.2	7.8	57.9	56.7	51.0	55.2
Aug. 9	36.2	37.3	40.7	40.9	8.3	7.4	54.1	54.2	52.6	55.6
Aug. 15	35.9	36.3	41.5	41.4	7.9	8.1	53.7	53.2	54.4	55.5

[1] Average of two varieties.
[2] N = normal soil moisture, D = below normal soil moisture.

and were slightly but not significantly higher in plants grown under dry conditions. A study by Sosluski et al. (1960) showed little or no increase in lignin content of intermediate wheatgrass varieties after the heading stage of maturity. This did not occur in the present study as ADL increased through the August sampling dates which were about six weeks after heading occurred.

IVDMD and IVCD both decreased significantly ($P<.01$) with later sampling date and reflected changes in chemical composition. Differences between intermediate wheatgrass plants grown on normal and dry soils were smaller than for bromegrass or crested wheatgrass. IVDMD means were 64.9 and 65.6% for normal and dry soils, respectively, while IVCD means were 63.6 and 64.4%, respectively.

The smaller differences in chemical composition and in vitro digestibility between intermediate wheatgrass grown on normal or dry soil may have been due to its later maturity. Intermediate wheatgrass did not reach the heading stage of maturity until June 16 while the other species headed within 2 to 3 days of June 3. The above normal rainfall in early and mid-June was apparently of more benefit to this species than the others. These observations were substantiated by yield data which showed no difference between normal and dry soil conditions for intermediate wheatgrass varieties, while yield of bromegrass and crested wheatgrass varieties were reduced by 32 and 39%, respectively.

Crested wheatgrass increased significantly ($P<.01$) in cellulose, ADF and ADL content with advancing maturity (table 25). Plants grown

TABLE 25. CHEMICAL COMPOSITION AND IN VITRO DIGESTIBILITY OF CRESTED WHEATGRASS AS AFFECTED BY SOIL MOISTURE SUPPLY[1]

Date	Cellulose		ADF		ADL		IVDMD		IVCD	
	N[2] %	D %	N %	D %	N %	D %	N %	D %	N %	D %
May 23	23.2	22.2	24.5	23.5	3.7	3.8	78.1	76.1	73.1	74.4
May 29	23.6	21.5	24.5	22.6	4.8	4.6	74.0	75.9	76.9	79.8
June 4	21.6	23.5	27.5	23.1	4.5	4.8	72.1	73.6	68.3	77.9
June 10	27.7	24.8	30.1	26.1	4.1	4.4	72.3	72.5	63.5	72.1
June 16	28.6	26.0	33.9	30.3	5.7	6.4	71.8	69.6	61.4	63.1
June 22	27.3	25.8	33.6	29.8	5.8	5.5	70.1	70.5	54.3	63.1
June 28	30.4	29.5	35.6	33.5	6.0	6.2	68.5	66.6	49.7	61.2
July 4	28.5	29.9	36.2	34.7	6.4	6.2	67.9	65.9	45.7	57.4
July 10	32.4	30.7	38.1	35.6	7.1	6.4	61.3	62.7	48.5	54.1
July 16	29.7	29.9	37.7	34.8	8.5	7.5	63.4	58.9	40.5	48.2
July 22	31.7	31.6	35.5	33.7	7.9	7.8	61.5	58.7	44.6	50.7
July 28	30.9	31.6	36.4	34.9	7.2	7.6	58.2	56.6	36.4	43.3
Aug. 3	33.7	32.9	36.9	35.9	8.1	8.3	56.7	55.2	43.4	41.7
Aug. 9	32.3	33.7	38.1	37.5	9.6	9.6	54.2	52.8	39.9	48.5
Aug. 15	34.2	34.1	38.3	37.1	8.7	8.6	53.2	52.5	48.1	51.9

[1] Average of two varieties.
[2] N = normal soil moisture, D = below normal soil moisture.

on dry soil contained significantly ($P < .01$) less cellulose than those grown on normal soils; however, the difference was small, 28.5% compared to 29.1%. Although the seasonal mean differed significantly, cellulose content was similar in late July and early August.

ADF content of crested wheatgrass from dry soils was 6.5% lower ($P < .01$) than from soils of normal moisture content. Unlike cellulose content which was similar late in the season, ADF content of grasses from normal soils remained higher throughout the season. Mean ADF content of crested wheatgrass was 33.8% for plants from normal soils and 31.6% for plants from dry soils.

As in the other species ADL content was not affected by soil moisture but did increase significantly ($P < .01$) with later sampling date. ADL content of crested wheatgrass was higher than the other two species studied.

IVDMD and IVCD both declined significantly ($P < .01$) with later sampling date, with the greatest decline occurring during the heading stage of maturity. The rapid decline in IVCD values for samples from dry soils occurred about one week later than in those grown on normal soils. IVDMD was significantly ($P < .01$) greater for samples grown on dry soils, 64.5% compared to 62.4%, than samples grown on normal soils. IVCD was also greater for plants grown on dry soils, 59.2% compared to 52.9%, a difference of 10%. This highly significant increase in IVCD could not be explained by lignification of the plants since ADL content did not differ significantly due to moisture conditions.

Variety by moisture within species means are presented in table 26. As shown both varieties of bromegrass, Greenar intermediate and Nordan crested wheatgrass all contained significantly more cellulose when grown on normal soils. Oahe intermediate and Siberian crested wheatgrass did not differ in cellulose content due to soil moisture. Manchar bromegrass contained significantly less cellulose than Sac at both soil moisture conditions. Greenar intermediate and Nordan crested wheatgrass contained more cellulose than Oahe intermediate and Siberian crested under normal moisture conditions but not under dry conditions. ADF content of all varieties was significantly higher at normal soil moisture levels than dry soil conditions. Manchar contained less ADF than Sac bromegrass under dry conditions. Greenar intermediate contained significantly more ADF than Oahe at both soil moisture levels, while Siberian contained less ADF than Nordan at normal soil moisture conditions.

ADL values did not differ significantly between soil moisture levels or varieties within species. IVDMD and IVCD values for all varieties except Greenar intermediate wheatgrass were significantly higher for plants grown on soils of below normal moisture content. Varietal differences in IVDMD and IVCD were small and are presented in table 26.

Species, moisture and species by moisture interaction means are presented in appendix table 4.

In summarizing the effects of soil moisture on chemical composition and in vitro digestibility of grasses, it was shown that

TABLE 26. MEAN CHEMICAL COMPOSITION AND IN VITRO DIGESTIBILITY OF GRASSES AS AFFECTED BY SOIL MOISTURE

	Bromegrass		Intermediate		Crested	
Moisture	Manchar %	Sac %	Greenar %	Oahe %	Siberian %	Nordan %
CMC						
Normal	28.7[a,1]	29.4[1]	30.2[a,2]	29.4	28.5[a]	29.6[1]
Dry	27.7[a]	28.3	29.2	29.4	28.6	28.4
ADF						
Normal	34.9[1]	34.6[1]	34.6[a,1]	33.8[1]	32.9[a,1]	34.7[1]
Dry	32.5[a]	33.2	34.0[a]	32.6	31.7	31.4
ADL						
Normal	6.3	6.4	5.7	5.7	6.6	6.5
Dry	6.1	6.3	5.8	5.9	6.6	6.5
IVDMD						
Normal	59.1[a,1]	60.7[1]	65.2[a]	64.6[1]	62.6[1]	62.2[1]
Dry	62.3	52.5	65.5	65.7	64.1[a]	64.9
IVCD						
Normal	51.4[a,1]	54.8[1]	64.1[a]	63.2[1]	53.7[a,1]	52.2[1]
Dry	56.6	56.8	64.2	64.6	58.1[a]	60.3

[a] Means differ between varieties within species ($P<.01$).
[1] Means in a pair differ significantly ($P<.01$) due to moisture condition.

plants from soils of below normal moisture content contained less cellulose and ADF than plants from soils of normal moisture content. ADL content was not affected by soil moisture levels. IVDMD and IVCD values were significantly higher for plants grown on soils of below normal moisture content.

The beneficial effects of lower cellulose and ADF content and slightly higher in vitro digestibility of plants was certainly overshadowed by the detrimental effect of lower dry matter yields per hectare. Although yields of intermediate wheatgrass varieties were not different due to moisture level, chemical composition was affected even though rainfall in June appeared to compensate for any previous moisture deficit.

The results of this study indicated that climatological factors influence the composition and in vitro digestibility of grasses and these factors along with differences due to species, varieties, cutting date and preparation methods all influence the quality of forage produced.

SUMMARY AND CONCLUSIONS

A study was made to determine if *in vitro* methods of determining digestibility could be used as a selection criterion by the plant breeder to predict animal utilization of forages. *In vivo* (conventional digestion trial) and *in vitro* comparisons were made to determine if differences in digestibility existed between varieties of three species of grasses when harvested at 50% head emergence and 14 days thereafter. *In vivo* digestibility differences between varieties within a species were small and nonsignificant. However, intermediate wheatgrass varieties differed significantly when closely controlled *in vitro* techniques were used. Animal variability reduced the probability of showing significance *in vivo* when small numbers of sheep were used. *In vitro* techniques were at least as effective as *in vivo* methods in evaluating relative differences in digestibility as evidenced by the highly significant correlations between the methods. IVDMD using the two stage technique could certainly be included in plant selection programs designed to develop superior varieties of forage.

The highly significant decline in crude protein and increases in fiber and lignin along with the highly significant decline in digestibility clearly point out that these grasses should be harvested on or before the date of 50% head emergence. This also infers that selection programs should include multiple collection dates. This became readily apparent when chemical analyses and *in vitro* digestibility determinations were made on forage varieties harvested throughout the 1966 and 1967 growing seasons. This phase of the study

was conducted to observe the rate of maturation of forage varieties to determine if varietal differences are maintained throughout the season. The results showed that cellulose content, ADF and ADL all increased significantly with later sampling date and that the increases were greatest during the heading stage of maturity. As a result of the increasing level of fibrous components and lignin, *in vitro* cellulose and dry matter digestibility declined significantly. Differences between varieties within species were small, although significant in some instances. In several instances one variety was not superior to another at all sampling dates. These differences may not have been noted with smaller numbers of sampling dates as demanded when *in vivo* trials are used. Species differences also existed; however, they could be attributed to maturity differences.

In order to determine the effects of anatomical changes on chemical composition and *in vitro* digestibility of grasses, plants were separated into leaf blade, leaf sheath, stem and seedhead fractions. It was thought that the determination of digestibility of individual plant fractions could provide useful information for plant selection work. It was found that leaf tissue decreased markedly as a result of heading while stem tissue increased most rapidly during this period. Leaf tissue declined nearly two percentage units per day while stem tissue increased the same amount as a result of heading. Leaf sheath was highest prior to stem elongation and decreased gradually thereafter. Seedheads composed as much as 30%

of the total plant dry matter shortly after heading but decreased to 10 to 15% late in the season.

Leaves contained less fiber and lignin and were more digestible than the other fractions with few exceptions while stems contained the most fiber and lignin and were lowest in digestibility. Leaf sheath values for fiber, lignin and digestibility were intermediate between leaves and stems but resembled stems more closely than leaves. Seedheads were variable in chemical composition and digestibility apparently due to seed development followed by shattering of the mature seed. The results of this study indicated that the amount of stem tissue present does not seriously affect digestibility of the whole plant when grasses are harvested at or before head emergence. Delaying harvest beyond this date decreases digestibility markedly due to the large increase in stem tissue and the low digestibility of this tissue late in the growing season.

Selection by the plant breeder for forage varieties containing stems and sheaths of high digestibility would appear to be more meaningful than selection for leafiness since these two fractions compose nearly 70% of the plant dry matter shortly after heading. This was evidenced by the lower digestibility of intermediate wheatgrass varieties which contained a greater percentage of sheath and stem tissue as compared to the other species.

A study was conducted in 1967 to determine the effects of below normal soil moisture on chemical composition and in vitro digestibility of grasses. It was found that plants grown on dry soils contained less

cellulose and acid-detergent fiber and had higher *in vitro* cellulose and dry matter digestibility values than plants grown on soils of normal moisture content. The beneficial effects of lower fiber and higher digestibility were overshadowed by reduced yields. This also indicates another area which cannot be overlooked by the plant breeder in his efforts to eliminate experimental error.

In conclusion, the data obtained in these studies showed that IVDMD would be of most benefit to the plant breeder for use in selection of plants with superior digestibility. The use of ADF and ADL are also recommended since correlations between these values and digestibility were high. Finally, the selection of plants having stems which remain high in digestibility during later stages of maturity is also recommended. The use of either of these criteria or a combination of the above used in association with present selection methods should provide for more rapid progress in the development of superior forage varieties.

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APPENDIX

TABLE 1. EFFECT OF YEAR OF HARVEST AND SAMPLING DATE ON CHEMICAL COMPOSITION AND DIGESTIBILITY OF GRASSES[1]

	1966					1967				
Date	CMC[2] %	ADF %	ADL %	DMD %	CD %	CMC %	ADF %	ADL %	DMD %	CD %
5-23	20.7	20.6	1.9	79.7	85.9	22.8	24.1	3.5	75.1	74.0
5-29	22.5	22.9	2.5	79.3	82.5	22.6	24.4	3.9	74.2	77.0
6-4	24.4	26.4	3.5	76.6	79.4	22.3	26.4	4.5	72.1	71.4
6-10	24.8	26.6	3.0	76.4	80.3	26.8	29.5	4.5	69.7	68.4
6-16	27.8	30.2	4.0	72.7	73.8	27.9	32.5	5.6	67.3	64.3
6-22	30.6	34.3	4.8	66.9	66.4	28.4	33.8	5.6	65.2	58.9
6-28	33.1	37.8	5.9	65.3	62.3	31.2	35.5	5.7	64.5	56.6
7-4	32.8	38.5	6.5	59.3	53.2	30.0	36.2	6.2	64.5	52.8
7-10	32.4	38.4	5.5	58.9	53.2	30.8	38.2	7.0	59.3	50.2
7-16	32.5	39.2	6.1	57.8	52.7	29.9	37.6	7.4	57.5	47.3
7-22	32.5	38.7	6.6	59.6	54.1	31.5	37.2	6.9	56.6	43.1
8-3	32.9	40.0	6.3	56.5	48.3	34.4	38.8	7.5	53.8	44.1
8-9	34.3	40.9	7.1	56.2	46.7	34.4	40.2	9.0	51.3	43.4
8-12	35.1	42.1	7.7	54.8	50.4	34.9	40.8	8.7	51.0	49.9

[1] Average of 6 varieties, 2 bromegrass, 2 intermediate wheatgrass and 2 crested wheatgrass.
[2] CMC = Crampton and Maynard cellulose, ADF = acid-detergent fiber, ADL = acid-detergent lignin, DMD = *in vitro* dry matter digestibility and CD = *in vitro* cellulose digestibility.

TABLE 2. SUMMARY OF SPECIES AND YEAR EFFECTS ON CHEMICAL COMPOSITION AND IN VITRO DIGESTIBILITY OF GRASS VARIETIES

	CMC[1] %	ADF %	ADL %	DMD %	CD %
		Species Means			
Bromegrass	28.2	33.6	5.6	63.1	57.5
Intermediate	30.4	34.8	5.4	65.6	65.5
Crested	30.4	34.6	6.2	62.7	55.8
		Year Means			
1966	29.9	34.4	5.2	65.2	62.7
1967	29.3	34.2	6.2	62.4	56.6
		Species x Year Interactions Means			
1966					
Bromegrass	27.4	32.4	4.8	66.2	61.9
Intermediate	30.9	35.4	5.1	66.2	67.5
Crested	31.7	35.5	5.8	63.1	58.6
1967					
Bromegrass	29.1	34.7	6.3	59.9	53.1
Intermediate	29.8	34.2	5.7	64.9	63.6
Crested	29.1	33.8	6.5	62.4	52.9

[1] See footnote appendix table 1.

TABLE 3. AVERAGE CHEMICAL COMPOSITION AND IN VITRO DRY MATTER DIGESTIBILITY OF PLANT FRACTIONS

Species component	Intact %	Leaf %	Sheath %	Stem %	Seedhead %
Bromegrass					
CMC[1]	28.9	24.5	36.1	38.3	26.6
ADF	32.4	30.7	39.3	41.4	31.9
ADL	6.5	7.1	6.8	7.1	7.0
DMD	63.7	65.9	54.8	50.5	55.3
Intermediate					
CMC	32.9	27.2	34.5	38.9	27.5
ADF	38.6	33.3	39.2	44.1	31.4
ADL	6.4	6.2	5.9	6.9	5.3
DMD	53.6	61.9	53.3	45.4	59.5
Crested					
CMC	32.4	26.6	36.2	39.3	27.9
ADF	36.7	32.4	39.8	42.7	30.3
ADL	6.9	7.6	6.7	7.4	6.6
AMD	58.7	59.5	54.5	51.2	60.7

[1] See footnote appendix table 1.

TABLE 4. EFFECTS OF SOIL MOISTURE ON CHEMICAL COMPOSITION AND IN VITRO DIGESTIBILITY OF GRASSES

	CMC[1] %	ADF %	ADL %	IVDMD %	IVCD %
		Species Means			
Bromegrass	28.6[a]	33.8[a]	6.3[a]	61.2[a]	54.9[a]
Intermediate	29.6[b]	33.8[a]	5.8[b]	65.2[b]	64.0[b]
Crested	28.8[a]	32.7[b]	6.5[a]	63.8[c]	56.1[c]
		Moisture Level Means			
Normal	29.3[a]	34.2[a]	6.2	62.4[a]	56.6[a]
Dry	28.6[b]	32.6[b]	6.2	64.2[b]	60.1[b]
		Species x Moisture Interactions Means			
Bromegrass					
Normal	29.1	34.7	6.3	59.9	53.0
Dry	28.0	32.9	6.2	62.4	56.7
Intermediate					
Normal	29.8	34.2	5.7	64.9	63.6
Dry	29.3	33.3	5.9	65.6	64.4
Crested					
Normal	29.1	33.8	6.5	62.4	52.9
Dry	28.5	31.6	6.5	64.5	59.2

[a,b,c] Means in a column differ at the 1% level of probability.
[1] See footnote appendix table 1.